



**INSTITUTE OF HEALTH  
FACULTY OF HEALTH SCIENCES  
SCHOOL OF PHARMACY**

**ETHNOMEDICINAL PLANTS FOR INFECTIOUS DISEASES IN  
ETHIOPIA: TRADITIONAL USE, PHYTOCHEMISTRY,  
ANTIMICROBIAL AND ANTIBIOTIC MODULATING EFFECTS,  
AND REGULATORY PERSPECTIVES**

**BY**

**SILESHI DUBALE DINSSA (B. PHARM. MSC)**



**AUGUST, 2024  
JIMMA, ETHIOPIA**

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**SILESHI DUBALE DINSSA (B. Pharm. MSc)**

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ETHIOPIA**

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## **DEDICATION**

This PhD dissertation is dedicated to everyone who has helped me reach the person I am today, especially my mother, Tayitu Nole (who died after I started my PhD program), and my father, Dubale Dinssa.

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## LIST OF ACRONYMS AND ABBREVIATIONS

|         |                                                     |
|---------|-----------------------------------------------------|
| AMR:    | Antimicrobial Resistance                            |
| ANOVA:  | Analysis of Variance                                |
| ASU:    | Ayurveda, Siddha, and Unani                         |
| AYUSH:  | Ayurveda, Yoga, Unani, Siddha, and Homeopathy       |
| BP:     | British Pharmacopeia                                |
| CC:     | Column Chromatography                               |
| CCIM:   | Central Counsel of Indian Medicines,                |
| CM:     | Conventional Medicine                               |
| COSY:   | Correlated Spectroscopy                             |
| CP:     | Conventional Pharmaceuticals                        |
| DAAD:   | German Academic Exchange Service                    |
| DACA:   | Drug Administration and Control Authority           |
| DEPT:   | Distortionless Enhancement by Polarization Transfer |
| DMSO:   | Dimethyl Sulfoxide                                  |
| EFDA:   | Ethiopian Food and Drug Authority                   |
| EPHI:   | Ethiopia Public Health Institution                  |
| FI:     | Frequency Index                                     |
| FIC:    | Fractional Inhibitory Concentration                 |
| GMP:    | Good Manufacturing Practice                         |
| HM:     | Herbal Medicine                                     |
| HMBC:   | Heteronuclear Multiple Bond Correlation             |
| IMS:    | Indian medicine system                              |
| IRB:    | Institutional Review Board                          |
| JuLaDQ: | Jimma University Laboratory of Drug Quality         |
| KI:     | Key informants                                      |
| LMICs:  | Low-and middle-income countries                     |
| MA:     | Market Authorizations                               |
| MIC     | Minimum Inhibitory Concentration                    |
| MM:     | Modern Medicine                                     |
| MoH     | Ministry of Health                                  |

|           |                                                                                                              |
|-----------|--------------------------------------------------------------------------------------------------------------|
| NDA:      | National Drug Authority                                                                                      |
| NEML:     | National Essential Medicine List                                                                             |
| NTD:      | Neglected Tropical DiseasesPlant-                                                                            |
| PBA:      | based antimicrobials                                                                                         |
| PV:       | Pharmacovigilance                                                                                            |
| RF:       | Retention Factor                                                                                             |
| RHR:      | Regional Health Regulatory                                                                                   |
| RHRA:     | Regional Health Regulatory Authority                                                                         |
| RMA:      | Resistance Modifying Activity                                                                                |
| SATCM:    | State Administration of Traditional Chinese Medicine                                                         |
| SDG:      | Sustainable Development Goal                                                                                 |
| SNNPR:    | South Nation Nationalities and Peoples Region                                                                |
| SOP:      | Standard Operational Procedures                                                                              |
| T&CM:     | Traditional and Complementary Medicine                                                                       |
| TCM:      | Traditional Chinese Medicine                                                                                 |
| TK:       | Traditional Knowledge                                                                                        |
| TLC:      | Thin Layer Chromatography                                                                                    |
| TM:       | Traditional Medicine                                                                                         |
| TMP       | Traditional Medicinal Practitioner                                                                           |
| TMPC:     | Traditional Medicine Practitioners Counsel                                                                   |
| UNESCO:   | United Nations Educational, Scientific and Cultural Organization                                             |
| USP:      | United States Pharmacopeia                                                                                   |
| V/V:      | Volume by volume                                                                                             |
| VDAFACA:  | Veterinary Drug and Animal Food Administration and Control Authority                                         |
| WHO:      | World Health Organization                                                                                    |
| YaBiNaPA: | Yaoundé - Bielefeld Bilateral Graduate School “Natural Products with Antiparasite and Antibacterial Activity |

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## TABLE OF CONTENT

|                                                                                            |       |
|--------------------------------------------------------------------------------------------|-------|
| Contents.....                                                                              | pages |
| ACKNOWLEDGMENTS .....                                                                      | VI    |
| LIST OF ACRONYMS AND ABBREVIATIONS.....                                                    | VII   |
| TABLE OF CONTENTS.....                                                                     | IX    |
| SUMMARY .....                                                                              | XIV   |
| CHAPTER I.....                                                                             | 1     |
| GENERAL INTRODUCTION .....                                                                 | 1     |
| 1.1. General Overviews of Infectious Diseases.....                                         | 2     |
| 1.2.1. Trends of Antibiotic Discovery .....                                                | 2     |
| 1.3.2. Ethnomedicinal Knowledge and Antibiotic Discovery from Medicinal Plants.....        | 3     |
| 1.3.3. Antimicrobial activity study of selected medicinal plant secondary metabolites..... | 3     |
| 1.3.4. Antibiotics and Plant Extract Combinational Effect.....                             | 6     |
| 1.3.6) Ethnomedicinal Use in Ethiopia.....                                                 | 7     |
| 1.4) Significance of the Study .....                                                       | 8     |
| 1.6. Outlines of the PhD Dissertation.....                                                 | 10    |
| 1.10. References .....                                                                     | 15    |
| CHAPTER II.....                                                                            | 21    |
| RESEARCH QUESTIONS AND OBJECTIVES .....                                                    | 21    |
| 2.1. Research Questions .....                                                              | 22    |
| 2.2. Objectives of the Study .....                                                         | 22    |
| 2.2.1. General Objective .....                                                             | 22    |
| 2.2.2. Specific Objectives .....                                                           | 22    |
| CHAPTER III: GENERAL METHODOLOGY.....                                                      | 24    |
| 3.1. Study Design and Period .....                                                         | 25    |
| 3.2. Systematic Literature Search.....                                                     | 25    |
| 3.2.1. Sources of Data and Selection .....                                                 | 25    |
| 3.2.2. Study included in Literature Searches .....                                         | 25    |
| 3.2.3. Literature Data Abstraction and Analysis.....                                       | 26    |
| 3.2.4. Literature Search of some WHO Member Countries TM Regulation Status .....           | 26    |
| 3.3. Ethnomedicinal Survey .....                                                           | 26    |

|                                                                                                                                  |    |
|----------------------------------------------------------------------------------------------------------------------------------|----|
| 3.3.1. Study Area .....                                                                                                          | 26 |
| 3.3.2. Study Settings .....                                                                                                      | 27 |
| 3.4. Ethnomedicinal Survey and Regulatory Framework Assessment .....                                                             | 28 |
| 3.4.1. Population.....                                                                                                           | 28 |
| 3.4.2. Inclusion and Exclusion Criteria .....                                                                                    | 29 |
| 3.4.4. Data Collection Instrument and Procedure.....                                                                             | 30 |
| 3.4.5. Validity and Reliability of Data Collection Tools .....                                                                   | 30 |
| 3.4.6. Criteria for Plant Material Selection.....                                                                                | 31 |
| 3.5. Materials, Chemical, and Microbial Strains.....                                                                             | 33 |
| 3.6. Extraction and fractionation of Plant Materials .....                                                                       | 33 |
| 3.7. Phytochemical Analysis .....                                                                                                | 34 |
| 3.8. Isolation of Pure Compounds .....                                                                                           | 36 |
| 3.9. Structural Elucidation.....                                                                                                 | 37 |
| 3.10. Antimicrobial Activity Tests.....                                                                                          | 38 |
| 3.11. Antibiotic Modulating Effect Assay.....                                                                                    | 39 |
| 3.12. Data Processing and Analysis .....                                                                                         | 40 |
| 3.13. Data Quality Control .....                                                                                                 | 40 |
| 3.14. Ethical Considerations.....                                                                                                | 40 |
| 3.15. Operational Definitions Some Terminologies.....                                                                            | 41 |
| 3.16. Dissemination of the Results.....                                                                                          | 41 |
| 3.17. References .....                                                                                                           | 42 |
| CHAPTER IV .....                                                                                                                 | 47 |
| ETHNOMEDICINAL PLANTS AND ASSOCIATED INDIGENOUS KNOWLEDGE FOR<br>THE TREATMENT OF DIFFERENT INFECTIOUS DISEASES IN ETHIOPIA..... | 47 |
| Abstract .....                                                                                                                   | 48 |
| 4.1. Introduction .....                                                                                                          | 49 |
| 4.2. Methods and Materials .....                                                                                                 | 49 |
| 4.2.1. Sturdy Design .....                                                                                                       | 49 |
| 4.2.2. Study Area and Period .....                                                                                               | 49 |
| 4.2.3. Data Collection Procedure .....                                                                                           | 49 |
| 4.2.4. A Literature Search Strategy .....                                                                                        | 50 |

|                                                                                                                                                 |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 4.3. Result.....                                                                                                                                | 51  |
| 4.3.1. Socio-Demographic Characteristics of TMPs .....                                                                                          | 51  |
| 4.3.2. Indigenous Designations of Suspected Illnesses by Traditional Medicinal Practitioners .....                                              | 53  |
| 4.3.3. Ethnomedicinal Plants used to Treat Infectious Diseases in Study area.....                                                               | 56  |
| 4.3.4. Ethnomedicinal Knowledge Transfer and Documentation Practices .....                                                                      | 67  |
| 4 5. Traditional Medicine Sources, Preparation, Storage, Accessibility, and Conservation                                                        | 69  |
| 4.3.6. Responses of TMPs on Future Activities Related to Ethnomedicinal Practices.....                                                          | 70  |
| 4.3.7. Comparative Analysis of Current Ethnomedicinal survey and Literature Reviews ....                                                        | 71  |
| 4.4. Discussion .....                                                                                                                           | 87  |
| 4.5. Conclusion and Recommendations .....                                                                                                       | 90  |
| 4.6. References .....                                                                                                                           | 91  |
| CHAPTER V .....                                                                                                                                 | 95  |
| PHYTOCHEMICAL SCREENING ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC<br>MODULATING EFFECT EVALUATION OF SELECTED MEDICINAL PLANTS IN<br>ETHIOPIA ..... | 95  |
| Abstract .....                                                                                                                                  | 96  |
| 5.1. Introduction .....                                                                                                                         | 97  |
| 5.2. Materials and Methods .....                                                                                                                | 97  |
| 5.3. Results .....                                                                                                                              | 98  |
| 5.3.1. The Ethnomedicinal Information of Selected Plants .....                                                                                  | 98  |
| 5.3.2. Phytochemical Screening.....                                                                                                             | 101 |
| 5.3.3. Antimicrobial Activities .....                                                                                                           | 103 |
| 5.3.4. Antibiotic Modulating effects of Selected Plants against <i>P. Eruginosa</i> (Atcc 27853)<br>And <i>S. Aureus</i> (Atcc43300) .....      | 107 |
| 5.4. Discussion .....                                                                                                                           | 111 |
| 5.5. Conclusion and Recommendations .....                                                                                                       | 113 |
| 5.5. References .....                                                                                                                           | 115 |
| CHAPTER VI .....                                                                                                                                | 121 |
| BIOASSAY-GUIDED ISOLATION AND STRUCTURAL ELUCIDATION OF POTENTIAL<br>ANTIMICROBIAL COMPOUNDS FROM <i>CRINUM ABYSSINICUM</i> ROOT BULB .....     | 121 |
| Abstract .....                                                                                                                                  | 122 |

|                                                                                                                                                                    |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 6.1. Introduction .....                                                                                                                                            | 123 |
| 6.2. Methods and Materials .....                                                                                                                                   | 124 |
| 6.2.1 , Plant Materials .....                                                                                                                                      | 124 |
| 6.2.2. Extraction and Isolation .....                                                                                                                              | 124 |
| 6.2.3. <i>In-vitro</i> Antibacterial Activity Tests .....                                                                                                          | 125 |
| 6.3. Result.....                                                                                                                                                   | 126 |
| 6.3.1. Isolated Pure Compounds and Elucidated Ion Structures.....                                                                                                  | 126 |
| 3.2. Antimicrobial Activity Test Results.....                                                                                                                      | 132 |
| 6.4. Discussion .....                                                                                                                                              | 134 |
| 6.5. Conclusion.....                                                                                                                                               | 135 |
| 6.6. References .....                                                                                                                                              | 136 |
| CHAPTER VII.....                                                                                                                                                   | 140 |
| TRADITIONAL MEDICINE REGULATORY FRAMEWORK AND LEGAL BASIS IN<br>ETHIOPIA: A CRITICAL EVALUATION OF CHALLENGES AND OPPORTUNITIES FOR<br>POLICY IMPLEMENTATION ..... | 140 |
| Abstract .....                                                                                                                                                     | 141 |
| 7.1. Introduction .....                                                                                                                                            | 142 |
| 7.2. Methods and Materials .....                                                                                                                                   | 143 |
| 7.2.1. Study Design and Period .....                                                                                                                               | 143 |
| 7.2.2. Study Area .....                                                                                                                                            | 143 |
| 7.2.3. Data Collection Procedure .....                                                                                                                             | 144 |
| 7.2.4. Data Analysis and Presentation .....                                                                                                                        | 144 |
| 7.3. Results .....                                                                                                                                                 | 145 |
| 7.3.1. Archival Review and Literature Search.....                                                                                                                  | 145 |
| 7.3.2. Socio-demographic Characteristics of Respondents.....                                                                                                       | 147 |
| 7.3.3. Availability of TM Product Regulation Systems and Frameworks .....                                                                                          | 147 |
| 7.3.4. Functionality of TM Products Regulatory Systems at The Federal Level.....                                                                                   | 148 |
| 7.3.5. Availability and Functionality of TM Practice and Providers' Regulation Systems by<br>RHRA .....                                                            | 149 |
| 7.3.7. Challenges and Opportunities for TM Regulatory Implementation .....                                                                                         | 151 |
| 7.4. Discussion .....                                                                                                                                              | 154 |
| 7.5. Conclusion and Recommendations .....                                                                                                                          | 157 |

|                                                                                      |     |
|--------------------------------------------------------------------------------------|-----|
| 7.5. References .....                                                                | 159 |
| CHAPTER VIII .....                                                                   | 166 |
| GENERAL DISCUSSION .....                                                             | 166 |
| 8.1. General Discution.....                                                          | 167 |
| 8.2. General Conclusion .....                                                        | 170 |
| 8.2. Implication for the Findings .....                                              | 173 |
| 8.3. Limitations of the Study .....                                                  | 175 |
| 8. 4. Recommendations .....                                                          | 175 |
| 8.5. Broader International Contecst, Relevance and Future Research Prospective ..... | 177 |
| 8.6. References .....                                                                | 179 |
| Curriculum Vitae of the Author .....                                                 | 184 |
| Signature snd Certification .....                                                    | 186 |
| Lists of Publications.....                                                           | 187 |
| Lists of PhD Candidate’s Training and Certificates .....                             | 188 |
| Anexs .....                                                                          | 193 |
| Annex -1. English and Afaan Oromo Verton of Questioners .....                        | 193 |
| Annex 2 NMR Spectras.....                                                            | 204 |
| Annex III: Different Certificates .....                                              | 216 |
| Annex V: Plagiarism checker Certificate.....                                         | 221 |

## SUMMARY

Plant-based natural products are crucial in healthcare; Secondary bioactive compounds from plants have diverse antimicrobial activities, and no reports of bacteria developing resistance. Ethiopia's medicinal plant resources are abundant, but a comprehensive database is lacking, antimicrobial activity, phytochemical screening, and active pure component isolation are unsatisfactory. And the practical implementation of traditional medicine (TM) regulation in Ethiopia remains unknown. Therefore, this PhD study aims to gather and document ethnomedicinal information, conduct phytochemical screening, and investigate antimicrobial activities, and antibiotic modulation effects of selected medicinal plants, as well as explore the regulatory implementation status of traditional medicine in Ethiopia.

**Chapter IV** presented the collection, documentation, and generation of evidence on the ethnomedicinal plants used and related indigenous knowledge for the treatment of perceived infectious diseases. Among 164 traditional medicinal practitioners interviewed, most of them replied that ‘*Dhukkuba dhiiraa/ye wend beshita*’ (male disease), sexual transmitted infections), *Ija namaa* (evil eye), and michii/sudden onset of respiratory tract illness and allergic reactions, were the most common illnesses. Seventy medicinal plant species belonging to 40 plant families were identified from the ethnomedicinal survey, of which 30 (44.9%) were newly reported for their claimed use against respective infections in the study area. Asteraceae is the most widely reported plant family. *Vernonia amygdalina* Delile was the most frequently used plant species. Leaves were the leading plant parts used, and oral route was the most frequently used route of administration. Intestinal parasites were the most common infections for which these top-listed plants were traditionally used. Consumer trust in traditional medicine was the primary reasons for community preference of traditional medicine practices. Traditional medicine practitioners primarily learn from their family members and share their knowledge verbally only with the selected offsprings. The majority of treatment approaches are plant-based, and they collect these plants from wild forests with difficulty of accessibility due to deforestation and over use.

**Chapter V** aimed to conduct phytochemical screening, antimicrobial activity, and

antimicrobial modulating effects on selected traditionally used medicinal plants. Accordingly, flavonoids, alkaloids, and phenols were the most abundant phytochemical constituents identified. The crude extracts and chloroform fractions of the extracts showed better activity against the tested microbial strains. The crude extract of *Thalictrum rhynchocarpum* Quart.-Dill. & A. Rich root demonstrated superior activity against all the tested strains, with the lowest minimum inhibitory concentrations of 0.48 µg/ml against *Staphylococcus aureus* and *Escherichia coli*. Moreover, Amoxicillin activity was increased by 8-fold (62.5 µg/ml) than its single effect when combined with *Crinum abyssinicum* Hochst. ex A. Rich against *Pseudomonas aeruginosa* (ATCC 27853). The antimicrobial plant extract had a promising fractional inhibitory concentration (FIC) index.

**Chapter VI** aimed to isolate an active anti-microbial compound from the root bulb of *Crinum abyssinicum*. The plant material was extracted using chloroform/methanol (1:1 v/v). Isolation of pure compounds was carried out using column chromatography over silica gel and gel filtration over Sephadex LH-20. Structural elucidations of isolated compounds were carried out using NMR spectroscopy. From the chloroform fraction, which exhibited low minimum inhibitory concentration, three compounds were isolated as Hippadine (compound **1**), 2, 3, 19, 23-tetrahydroxy-urs-12-en-28-oic acid (compound **2**) and 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (compound **3**).

**Chapter VII** evaluated the traditional medicine regulation legal basis and its practical implementation in Ethiopia. The study revealed that Ethiopia is regulating traditional medicines based on the Medicine Policy of 1993 and the Food and Drug Authority of Ethiopia's Proclamation No. 1112/2019. About 70% of federal regulatory officers and 41.7% of regional regulatory officers responded that a traditional medicine regulatory activity in Ethiopia is weak. Barriers to proper TM regulation include poor governance, resource limitations, lack of stakeholder cooperation, and weak legal enforcement. The current Ethiopian traditional medicine regulation framework is inadequate to ensure quality, safety, efficacy, and rational use of traditional medicine.

**Chapter VIII** presents the general discussion of the study, implication of the findings and future perspectives.

**Keywords:** Indigenous knowledge, primary health care, medicinal plants, phytochemical, antimicrobial activity, regulation.





## **CHAPTER I:**

### **GENERAL**

### **INTRODUCTION**

## **1.1. General Overview of Infectious Diseases**

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi, or parasites and can be transmitted from person to person. Globally, infectious diseases are the leading cause of premature death and disability. Particularly in developing countries, it affects the poorest and also contributes to poverty (1,2). Among these microbial infections, bacteria continue to pose the greatest risk to human health. Bacterial infections accounts for more than 13% of deaths. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* are among the most responsible for more than half of cases. Preventing and treating microbial infections requires multidisciplinary techniques, including the development of vaccines and antibiotics(3–6).

The significant progress in the development of antimicrobial agents for clinical use was short-lived. The efficacy of many of these early antibacterial medicines is now reduced due to the development of resistance (2,7–9). Bacteria can develop resistance to antimicrobials through different mechanism including selective pressures or acquisition from neighboring microbes, intrinsic resistance due to lack of target site, pharmacological incapacity, or chromosomal-encoded resistance mechanisms (2,10,11).

Both microbial infections and antibiotic resistance double the burden of health care, causing millions of deaths annually. The rapid spread of these issues raises concerns about treating infectious illnesses. Currently, we are in a post-antibiotic world in which ordinary diseases and mild injuries might kill. It is not an apocalyptic narrative but rather a very plausible potential for twenty-first century health care (3,7,11). Drug-resistant microbial infections cost hospitals more than non-resistance infections, causing 400,000 cases, 25,000 deaths annually, and €1.5 billion in losses. This uncertain threat impacts human health, livestock production, and other unforeseen effects (12–14).

## **1.2. Theoretical Framework of the study**

### **1.2.1. Trends of Antibiotic Discovery**

The discovery of penicillin in early twentieth century transformed the treatment of infectious illnesses. It marked the beginning of a new age of microbial illness treatment. Since then, there was an exponential growth in the number of antibiotics discovered (2,15,16). Many antibiotics have been produced as a result of the synthetic tailoring of these scaffolds. Antibiotic development, primarily by focusing on microbial sources or synthesizing new

compounds using existing antibiotic scaffolding methods, is declined due to bacterial resistance. Food and Drug Authority approval for new antimicrobial agents has debilitated by over half in the last three decades, leading to a decrease in the availability of new, and effective antibiotics (11, 17).

The current development of new antibacterial medicines is insufficient and the antibacterial clinical and preclinical pipeline is slow. Yet, the rate and success of innovation are much below what is required to secure contemporary medicine's benefits. Urgent and concerted investments in research and development are therefore needed to accelerate and expand the pipeline for antibiotics (18–20). The expense of antimicrobial agent research and development has escalated to a level that yields poor profitability for the pharmaceutical markets. This is worsened by the length of time necessary for new drugs to pass clinical tests (2,9,21,22).

### **1.3.2. Ethnomedicinal Knowledge and Antibiotic Discovery from Medicinal Plants**

Traditional knowledge (TK) is a complex concept that encompasses various subjects. It can exist in indigenous or local communities as a secret oral tradition. Ethnomedicinal information is crucial for systematically observing traditional medicinal plants and supporting claims for therapeutic products. Evidence should be obtained from the appropriate TMP or indigenous group, considering theories, concepts, cultural context, plant species, part of the plant used, season of plant collection, and stage of plant development. However, most of this knowledge is not revealed in scientific studies (23,24,24).

Plant-based TM systems continue to play an important role in the healthcare system. Around 80% of rural populations in developing countries rely on TM for primary healthcare need. According to WHO report, over 33% of pharmaceuticals produced by industrialized nations are plant-derived. Over 20,000 medicinal plant species were estimated for potential applications. However, most of these plants have not been adequately evaluated (20,25,26). Medicinal plants have enormous potential for the discovery of new bioactive compounds that can fight against resistant microorganisms with a high concentration of active chemicals with antibacterial action (2,22,27,28)

### **1.3.3. Antimicrobial activity study of selected medicinal plant secondary metabolites**

Plant secondary metabolites are primarily responsible for their antibacterial properties. Major

classes of phytochemicals with antimicrobial activity are phenolics and polyphenols (flavonoids, quinones, tannins, and coumarins), terpenoids, alkaloids, and polypeptides. The processes of medicinal plants include: collection, authentication, drying, grinding, extraction, fractionation, and isolation of bioactive compounds and the choice of these methods may determine the efficacy of the tested medicinal plants (29–31).

The secondary metabolites with biological activity are also influenced by factors like physiological variances, environmental conditions, regional variations, genetics, and plant material quantity. They have bioactivities like antibacterial, anti-inflammatory, and antioxidant properties (32–34). Despite the growing number of compounds isolated from these antimicrobial medicinal plants, there are few plant-derived drugs in clinical use due to the complexity of the extract composition. Synergistic, additive, and antagonistic effects can result from the combined effects of plant extracts, making it challenging to classify and identify contributing compounds. While bacteria may not develop resistance to botanicals, they may develop resistance if only one active principal is targeted. Similarly microbial communities' diversity, structure, size, and density also influence interactions within these tested plant extracts (34–37).

Plant-derived secondary bioactive compounds contain complex ingredients that affect microbial cells through cytoplasmic membrane structure, integrity, and functionality. Some compounds modulate the immune response, mitosis, and apoptosis. There are no reports of bacteria developing resistance to plant-based antimicrobials (PBAs). However, only a few herbal medicines have been examined.(2,38,39). The following figures illustrate some antimicrobial compounds previously isolated from plants, as evidenced from different literatures (Source 2,38,39),

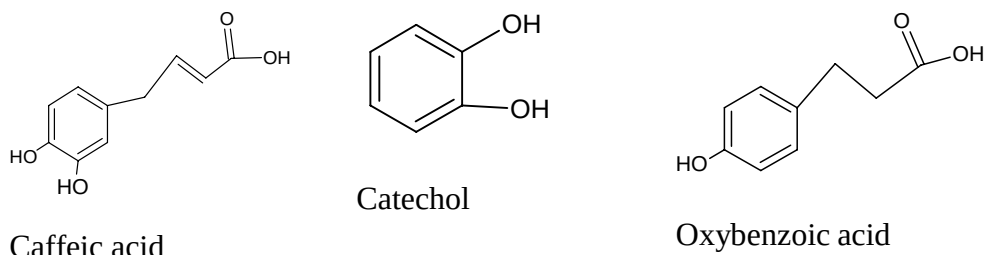
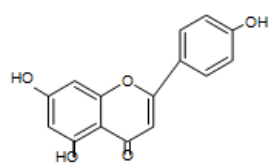
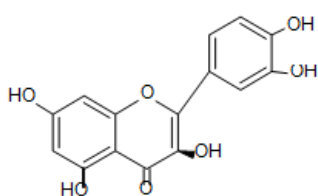


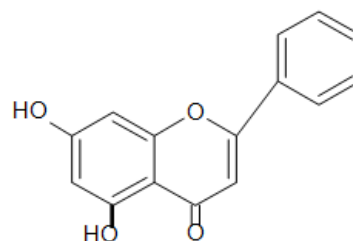
Figure 1. 1A: Some phenolic derivatives from plant extracts



Filavone

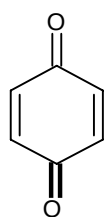


Catechin



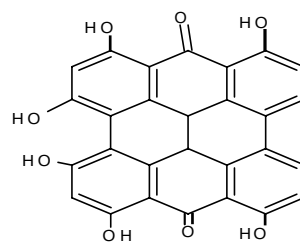
Chrysin

Figure1.1B. Some flavones and flavonoids antibiotic compounds derived from plant extracts



Quinon

e



hypericin

Figure 1.1C: Quinone and hypericin, compounds derived from plant extracts

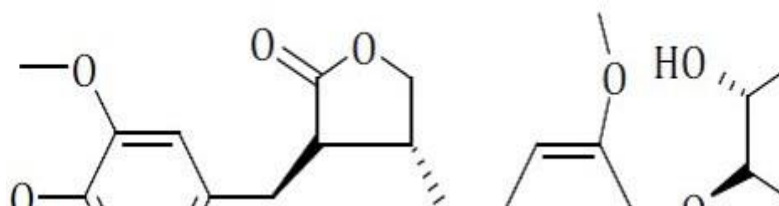


Figure 1.1 D. Styrylflavonoid compound derived from plant extracts

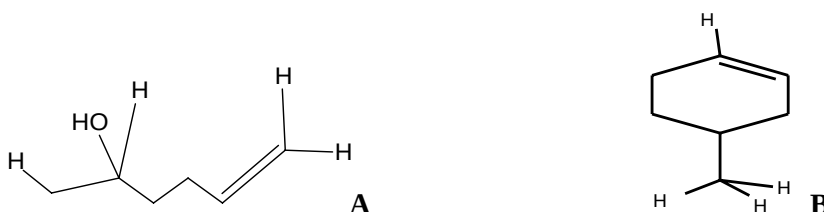


Figure 1.1E Chemical structure of a) linalool; b) limonene; compounds derived from plant extracts,

### Antibiotics and Plant Extract Combinational Effect

Combination therapy has gained popularity in infectious disease treatment. The WHO prefers combination therapy over monotherapy for life-threatening diseases like malaria, tuberculosis, and HIV/AIDS. Combination therapy aims to target multiple disease facets and reduce resistance, making it a research priority due to its cost-effectiveness and the potential to restore existing drugs (11,40,41).

Synergistic interaction between plant extracts and conventional antibiotics could be a potential effective method for combating antibacterial resistance. Studies show that plant extracts can reduce antibiotic MIC against resistant strains. A process known as resistance-modifying activity (RMA) can help to restore the clinical application of obsoleted or going to be obsoleted antibiotics by increasing their potency. It can expedite clinical usage by reducing development costs, offer increased efficiency, reduced side effects, good stability, high bioavailability (34,37). As evidenced from different literature, the "repurpose" of conventional antibiotics could significantly impact global health by combating resistant

pathogenic microorganisms (42–45). The number of studies addressing the synergistic interaction between plant extracts and resistance-prone drugs has dramatically expanded during the previous decade (4,57–59). But this evidence is lacking in Ethiopia, and there is also no legal background to use conventional drugs in combination with medicinal plant extracts.

#### **1.3.4. Regulatory Aspects of Traditional Medicines**

Ensuring safety, quality, and efficacy of TM remains a global challenge. Developing methods to improve TM availability is crucial for accessing excellent healthcare. Despite efforts, the availability of safe, high-quality, effective, and reasonably priced TM remains unappealing (53, 60–62). For example, the widespread use of *Crotalaria* in treating measles can result in the emergence of liver lesions in children in East Africa, and the broad use of *Crotalaria*, *Cynoglossum*, and *Scenecio* has been linked to liver and kidney illness in Ethiopia (51–53).

In many countries, TM products and practices is not regulated. Most TM products used often lack safety and efficacy criteria, leading to hidden morbidity, mortality and poor attention from pharmaceutical companies, particularly in sub-Saharan countries. To address these concerns, a competent regulatory structure is needed. The legal framework is crucial for promoting safety, effectiveness, and quality of TM products and practices (48,54,55).

#### **1.3.6) Ethnomedicine Use in Ethiopia**

In Ethiopia, it is believed that 70% of the human population and 90% of the livestock population rely on medicinal plants for primary health care. Approximately, it is estimated that 6500-7000 species of higher plants are available and 12% of them are endemics. However, research on bioactivity, phytochemicals is minimal. Safety and efficacy of these TMs are often uncontrolled. Current research mainly consists of surveys, with incomplete descriptions and uncertain recipes for plants used as remedies. Knowledge is also passed down orally from generation to generation (56,57).The therapeutic and toxic effects of TM have not been adequately investigated. Integrating TM into the formal health system has not been addressed (58–60). There is no rigorous research related to the bioactivity, phytochemistry, and regulation of TM in Ethiopia. Ethiopian people believe that all TM are harmless because they are natural. But some TM plants are well recognized to be poisonous, and TMP may cause harm as well as create delays in the treatment of communicable diseases such as TB (54,60,61). The Ethiopian government is aiming to regulate TM practices to

ensure safety, efficacy, and quality. They have enacted a proclamation, "Food, Medicine, and Health Care Administration and Control Proclamation No. 661/2009. " There is no clear information about registered TMPs, regulatory requirements for manufacturing or safety assessment (62–64).

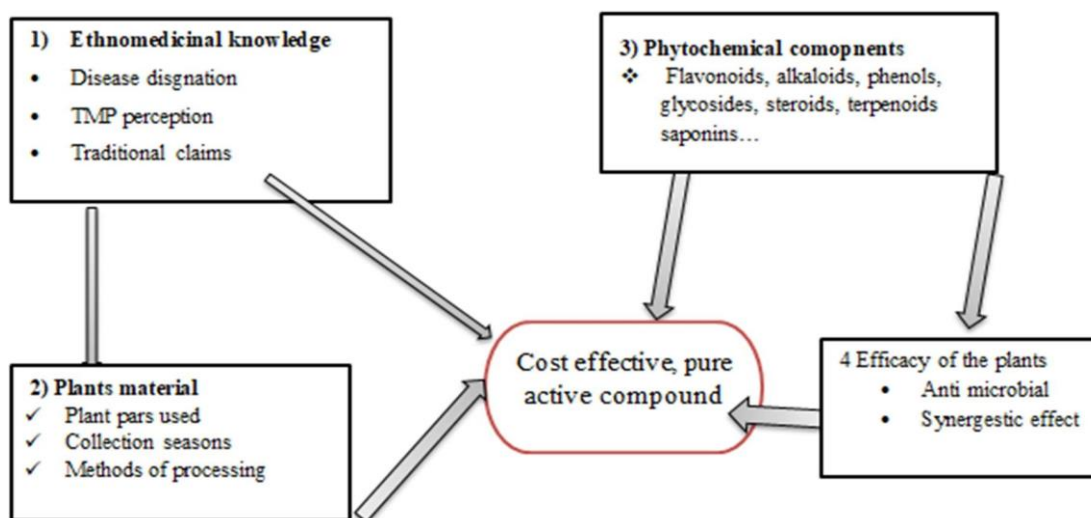


Figure 1.2: Simplified conceptual Framework model of the study (23,24)

#### 1.4) Significance of the Study

Traditional medicine (TM) has largely contributed to basic health care services across the world. In the African region, TM knowledge and practices have been contributing to the goal of access to basic health care. Plant materials have been used for medicinal purposes since ancient times, providing antibacterial chemicals against pathogenic diseases (51,56,65). Traditional medicine in Ethiopia, based on indigenous knowledge, is under threat due to factors such as old TMP disappearances, agricultural expansion, population dynamics, urbanization, environmental degradation, and overuse. Despite the abundance of ethnomedicinal plants, only small fractions are utilized. To address antibiotic resistance, new approaches are needed to fill the antimicrobial drug development pipeline. Innovative strategies, such as plant extracts combined with conventional antibiotics, may offer affordable treatment options, making antimicrobial natural product combination drugs a research priority due to their ~~own~~ advantage over conventional drug discovery methods (24,30,31).



To generate TM knowledge, the first and most important stage in a drug development is collection and analysis of information on the use(s) of the plant(s) by various indigenous cultures. Ethnomedicinal information can be used to 'pre-screen' plants for experimental pharmacological research (24,30). Selecting a reasonable number of plants to be tested and conducting bioassay systems that are believed to predict the action of these drugs in humans is crucial. Identifying the classes of active constituents present in medicinal plants and purifying this active compound is an important next step in pulling comprehensive scientifically sounded evidence about selected plants. Ineffective TM regulation can lead to substandard, fake, unsafe, and ineffective products and practices, posing serious health risks. Regular monitoring and evaluation of the TM process are critical to identifying legal gaps and ensuring compliance with legislation (29,31,57). There is a scarcity of a full-information database of documented medicinal plant species antimicrobial activity test findings, phytochemical screening, and the isolation of active pure components. The status of TM regulation's practical implementation is also unknown in Ethiopia (Reference).

Therefore, the present study focused on the collection and documentation of information related to indigenous knowledge and medicinal plants used for infectious diseases in Ethiopia; antimicrobial activity testing; phytochemical screening; the antibiotic modulation effect assessment of selected medicinal extracts with conventional drugs; and the assessment of practical TM regulatory implementation status in Ethiopia.

Thus, this study is a new approach that brings different TM research information into a single table. Gathering of information related to ethnomedicinal knowledge, bioactivity tests, molecular characterization, and regulatory status of TM, as well as plant extract and conventional antibiotic combination effect analysis were considered.

This research increased the country's database of medicinal plant species and related indigenous knowledge used for the treatment of infectious diseases. It provided fundamental information for research institutes, policymakers, and the pharmaceutical industry about the prospects of anti-microbial drug development from medicinal plants. It is the baseline for further research to conduct modern drug and plant extract effects for “re-storing” the activity of conventional antibiotics that are prone to obsolete to reach clinical usage much more rapidly and at a much lower development cost. It provided evidence for the generation of

knowledge

and skills to teach undergraduate and postgraduate students from different departments and to train TMP on identified skill gaps. It provided evidence about the practical implementation of the existing TM regulatory system that promotes safe, effective, quality TM knowledge, practice, and products in Ethiopian primary health care.

#### 1.6. Outlines of the PhD Dissertation

This dissertation contains eight major chapters that broadly identify indigenous knowledge and selected medicinal plants traditionally used for the treatment of perceived infectious diseases in Ethiopia. **Chapter I:** Introduces an overview of infectious disease problems, trends in antibiotic discovery, the theoretical framework of antibiotic discovery from plant extracts, the significance of the study and the outlines of the dissertation. **Under Chapter II:** the research questions and the objectives of the study were stated. **Chapter III:** focused on explanation of methodologies of the study

**Under Chapter IV:** Ethnomedicinal plants and associated indigenous knowledge used for the treatment of different infectious diseases in Ethiopia were assessed. Medicinal plant species used for treatment of infection by TMP were identified. The study identified the indigenous designations given by traditional healers in local language for different infectious diseases, including the description of the illnesses and how they perceive it. Traditional medicinal practitioners were also interviewed to generate information related to why communities prefer ethnomedicinal services to modern medicine, how TMP acquired TM knowledge and practices, to whom they transfer their knowledge and practices, and whether they document or not the services they provide. Medicinal plant sources, preparation methods, storage system, accessibility of medicinal plants, and conservation status were evaluated.

Details of the literature were also searched to gather evidence of medicinal plants utilized across the country for the treatment of common human infections and compared it with the current survey. Traditional medicine practitioners and communities in the study area have developed different knowledge and perspectives on how to designate different human infections based on different cultural and spiritual beliefs over time. Most of the TMP reported that they inherited their knowledge from family members and needed it to be orally transferred only to their children. The communities preferred ethnomedicine to modern

medicine because of its affordability, readily available, and perceived freedom from adverse effects. The documentation of the service provided by TMP was almost negligible. The majority of TMP reported that they collected medicinal plants from the wild, with difficulties in obtaining these plants because of deforestation and agricultural expansion. A few TMP conserve the plants in their home gardens, and very few control and protect the massive harvesting and degradation of wild medicinal plants. From 70 medicinal plant species belonging to 40 plant families identified by the ethnomedicinal survey, thirty of them were newly reported and identified for their use against perceived infections in the study area without any evidence from a literature search.

**Chapter V:** Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants were determined. Plant species were selected for antimicrobial activity and phytochemical screening by setting different criteria. These criteria include the ease of accessibility of the plants, traditional claims, TLC spots and separations, and some literature evidence. Based on the information gathered from the criteria, twelve plant species were identified, including: *Justicia schimperiana* (Hochst. ex Nees) root; *Croton macrostachyus* Hochst. Ex Delile stem bark; *Albizia gumifera* (J.F.Gmel.) C.A. Sm. stem bark; *Clematis hirsuta* Guill, and Perr whole part; *Solanum nigrum* L. fruit; *Dodonaea angustifolia* L.f. leaf; *Crinum abyssinicum* Hochst. ex A. Rich root bulb; *Trichilia dregae* Sand, stem bark *Dracaena steudneri* Engl. Root; *Momordica foetida* Schumacher et Thonn Leaf, *Thalictrum rhynchocarpum* Quart.-Dill. & A.Rich root; *Pycnostachys abyssinica* Fresen root.

These plants were subjected to phytochemical screening and *in-vitro* anti-microbial activity tests against selected microbial strains. Minimum inhibitory concentrations were measured using the broth micro-dilution method. Most of the selected plant extracts presented notable positive phytochemical results with flavonoids, alkaloids, and phenols. Among the selected plant species, all fractions of the extracts from *T. rhynchocarpum* root presented the greatest efficacy against all tested strains. Extracts from *J. schimperiana* and *C. macrostachyus* also demonstrated the next remarkable activity against tested microbial strains. The chloroform fraction of *J. schimperiana* root presented the highest activity with lower MIC against *S. aureus* and *E. coli*. The crude extract of *C. macrostachys* exhibited high activity against *K. pneumoniae*, *S. aureus*, and *E. coli*. The chloroform fraction of *C. macrostachys* also demonstrated the lowest MIC against *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The

combinational or interactive inhibition was determined using the checkerboard method for measuring the synergistic antibiotic-modulating effects of plant extract. The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotics (Tetracycline, Amoxicillin, and Cloxacillin) with tree plant extracts having lost MIC (*C. abyssinicum* chloroform fraction with an MIC of 7.8 against *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 43300), and *C. hirsuta* with MIC of 7.8 against *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 43300), and *T. rhynchocarpum* crude extract with MIC of 0.98 against both above-selected bacterial strains. The FIC value for each agent was calculated using the formula  $FIC = MIC_{\text{Antibiotic in combination}} / MIC_{\text{Antibiotic alone}}$ . The MIC of all tested antibiotics is at least doubled the efficacy when combined with plant extracts. The amoxicillin activity was increased 8 times when combined with *C. abyssinicum* Hochst. ex A. Rich against *P. aeruginosa* (ATCC 27853).

**Chapter VI:** Based the information generated from chapter two and three, one medicinal plant species, *C. abyssinicum* Hochst. ex A. Rich root bulb were selected for column chromatography separation. It showed good TLC spot and remarkable separation; and was subjected to bio-guided isolation of pure compounds. Three compounds were isolated and its structure is elucidated. The plant material was extracted using DCM/methanol solvent systems. The crude extract was fractionated with n-hexane, dichloromethane, ethyl acetate, and methanol. Isolation of pure compounds was carried out using column chromatography over silica gel, gel filtration over Sephadex LH-20. Structural elucidations of isolated compounds were carried out using NMR spectroscopy. The first compound (**1**) was isolated as 12 mg of a white, powder with an R<sub>f</sub> value of 0.7 in a 7:3 n-hexane and ethyl acetate solvent system, and its chemical structure was hippadine. The second compound (**2**), 9 mg were isolated as a dark, white powder with an R<sub>f</sub> value of 0.52 in 2:8 n-hexane and ethyl acetate. It is 2, 3, 19, 23-tetrahydroxy-urs-12-en-28-oic acid. The third compound(**3**), was isolated as six mg a white crystal with an R<sub>f</sub> value of 0.45 in a 0.5: 9.5 n-hexane and ethyl acetate solvent system, which was verified to be 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one. All the three isolated compounds displayed moderate activity against tested strains. As an alternative, isolated compounds from *C. absnicum* could be a potential answer for the development of an active antibiotic for drug-resistant microbes and further studies also recommended.

**Chapter VII:** Traditional Medicine Regulatory Framework and its practical implementation in Ethiopia were critically evaluated. Through archival review, literature search and cross-sectional survey, the availability, functionality, the overall performance of the practical implementation, challenges and opportunities of TM product regulation systems and frameworks were assessed. TM regulation in Ethiopia is carried out in conjunction with allopathic medicine under proclamation No. 1112/2019. The definitions of TM practice and practitioner are not addressed in this proclamation. So far, there is no officially registered TM product by the federal regulatory authority, although some galenical preparations are available on the market. The registration of TMP is authorized by the regional health bureau. Curriculum development for education on TM is not yet nationally authorized or materialized. There is no health insurance coverage for TM healthcare provision at all. The code of ethics for TMP, the TM national expert committee, TM local production, national monographs for TM, the TM national pharmacopoeia, the NEML of TM products, the national plan for integrating TM into the national health service delivery, and related information are not available in Ethiopia's TM policy. The draft preparation of the TM policy has been completed, but it has not yet been endorsed. To safe life of public and provide safe, effective and quality TM for public health and to promote, commercialize and contribute for economic growth of the country a lot of gabs related to TM regulation were identified by this study and prospective curtail works should be addressed were recommended.

**Finally,** Chapter **VI:** presents the general conclusions, implication of the findings and future research perspectives. In summary, a graphical representation / a schematic of the different aspects investigated in this work is given in Figure 1.3.

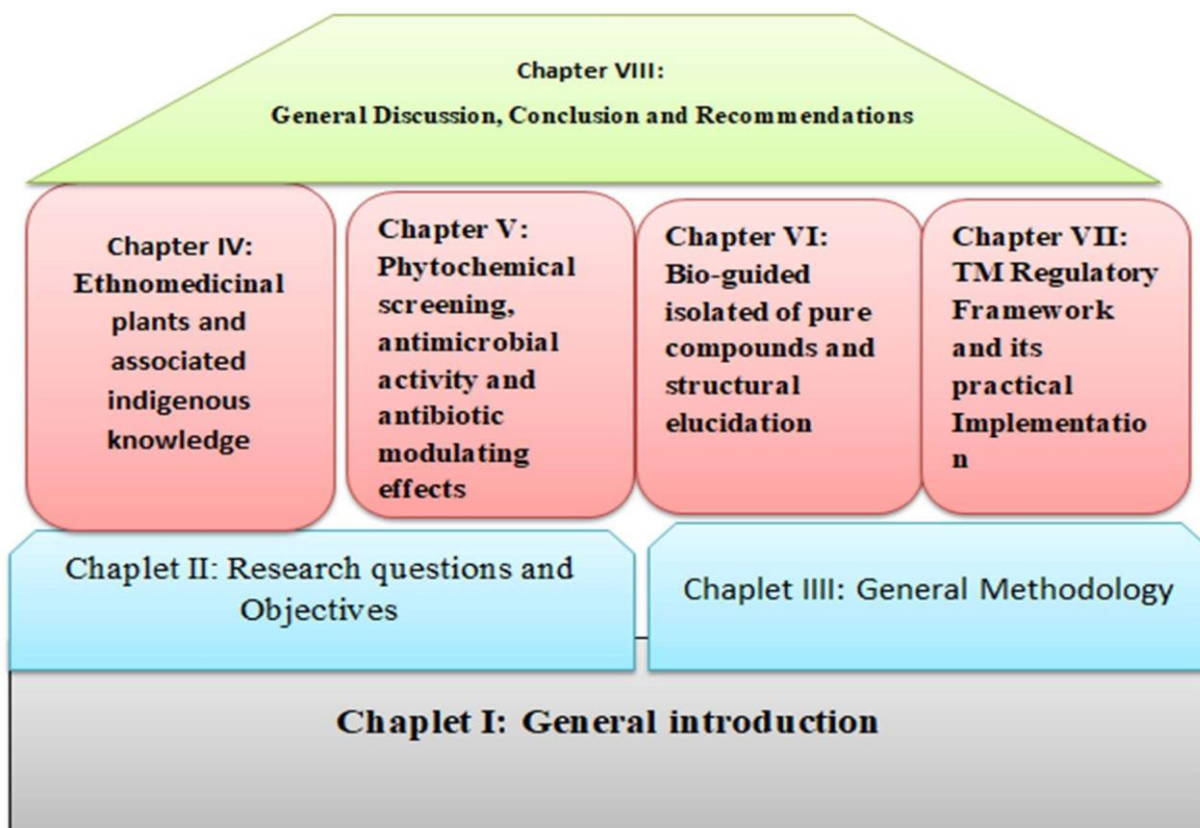


Figure 1.3: Graphical representation of the dissertation's outline.

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# 2

## **CHAPTER II: RESEARCH QUESTIONS AND OBJECTIVES**

## **2.1. Research Questions**

- 1) What is the ethnomedicinal knowledge related to medicinal plant potentials available for the treatment of infectious diseases in Ethiopia?
- 2) What types of bioactive phytochemicals are available in the selected medicinal plants?
- 3) What are the effects of combination of selected plant extracts with conventional antimicrobials against selected microbial strains?
- 4) What is the status of TM regulation in Ethiopia?

## **2.2. Objectives of the Study**

### **2.2.1. General Objective**

The general objective of this study was to assess indigenous knowledge related to ethnomedicinal plants used for perceived infectious diseases, conduct *in-vitro* antimicrobial activity tests, identify phytochemical components of selected medicinal plants, determine the antimicrobial modulating effects of selected medicinal plants against some selected bacterial strains, isolate active pure compounds and explore the regulatory status of TM in Ethiopia.

### **2.2.2. Specific Objectives**

- 1) To determine ethnomedicinal knowledge related to plants used for the treatment of different perceived infectious diseases in Ethiopia.
- 2) To assess the *in-vitro* antimicrobial activity of selected medicinal plants traditionally used for the treatment of perceived infectious diseases in Ethiopia.
- 3) To conduct preliminary phytochemical screening
- 4) To evaluate the antibiotic-modulating effects of selected active medicinal plant extracts against selected microbial strains.
- 5) To isolate pure active compounds from selected medicinal plant extracts
- 6) To assess the legal frameworks for TM in Ethiopia.



# 3

## **CHAPTER III: GENERAL METHODOLOGY**



### 3.1. Study Design and Period

The study design incorporated comprehensive literature review to collect detailed information about medicinal plants previously studied for utilization in different human infections; a cross-sectional ethnomedicinal survey to assess medicinal plants traditionally used and associated indigenous knowledge for the treatment of perceived infectious diseases (**Chapter IV**) (from January to September 2020), qualitative phytochemical screening, *in-vitro* antimicrobial activity test of selected medicinal plants (from 1<sup>st</sup> June to 31<sup>st</sup> September 2021) (**Chapter V**), isolation and structural elucidation of compounds from one selected plant (**Chapter VI**) and an institutional-based cross-sectional study of TM regulatory framework in Ethiopia ( (**Chapter VII**) (from November 2021 to March 2022)).

### 3.2. Systematic Literature Search

A literature search was conducted to identify medicinal plants traditionally claimed for the treatment of perceived infectious diseases in Ethiopia using different search engines. Information obtained from the literature review was used to validate the traditional claims about the use of medicinal plants for the treatment of infectious diseases.

#### 3.2.1. Sources of Data and Selection

A literature review was carried out through search in databases, including Medline/PubMed, Google Scholar, Science direct, HINARI and Embase. Keywords such as ethnomedicine, ethnopharmacology, ethnobotany, traditional medicine, herbal medicine, medicinal plants, antimicrobial activities of medicinal plants, indigenous knowledge, natural products, folk medicine, and home remedies were used. Unpublished thesis or dissertation reports were also included from repositories of different universities and by finding hard copies from library.

#### 3.2.2. Study included in Literature Searches

Articles that do not contain complete ethnomedicinal data, such as plant family, species names, those that do not address one or more infection types, were excluded from the review. The applicable articles were selected by using the following inclusion criteria: (1) at least one species of medicinal plant used in the treatment of perceived infection symptoms; (2) medicinal uses, parts used, growth habit, and route of administration; and (3) studies conducted in Ethiopia.

### **3.2.3. Literature Data Abstraction and Analysis**

The details of medicinal plants were extracted from each study using an abstraction form, including scientific names, family names, and local names; growth forms of the plants; plant parts used; methods of preparation; specific disease uses; route of administration; and whether bioactivity tests as well as phytochemical analyses were analyzed.

### **3.2.4. Literature Search of some WHO Member Countries TM Regulation Status**

The TM regulation status of all African countries, as well as India's and China's, was critically evaluated based on WHO TM regulation parameters. The availability, sufficiency, and comprehensiveness of Ethiopia's legislative framework for TM regulation were compared with those of selected African countries, as well as India and China. Countries were selected after a comprehensive review of the literature based on the 2019 WHO Global Report on TCM Checklist and the 2013 Traditional Medicine Strategy (2014–2023). Based on the checklist: three African countries with the highest performance: Ghana (90.9%), average performance Uganda (77.3%), and lowest performance Kenya (13.6%) were selected for comparison. India and China were purposively selected because they have strong TM regulation systems (1,2). The checklist used in the review process was revised and

## **3.3. Ethnomedicinal Survey**

### **3.3.1. Study Area**

#### **Country Profile**

Ethiopia is the second-most populous country in Africa, with a diversity of cultures and languages. Ethiopia's geographic area extends from the highest altitude (4,620 m above sea level) to 1500m below sea level. The population of Ethiopia is estimated be more than 120 million, which is characterized by a rapid growth rate of 2.6% per year. The country is characterized by a wide range of ecological and climatic conditions and a diversity of flora compositions. The flora of Ethiopia is estimated at 6500–7000 species, including medicinal plants of 12–19% that are endemic to the country (3,4). Ethiopia has a federal government administration system that is divided into 12 regional states and two city administrations. Further, each region is subdivided into zones, districts (Weredas), and kebele (local administrations). In Ethiopia, even though modern health care started before a century, the growth of health care sectors, pharmaceutical services, and drug access is very slow. Due to poor access to health services, especially in rural areas, the majority of Ethiopians rely mainly

on TM for their primary health care needs (5).

### **3.3.2. Study Settings TM Legal framework assessment**

The ethnomedicinal study was conducted in Southwest Ethiopia. Three zones of the Western Oromia regional state including Ilubabor, Jimma, and Kelem Welega; two neighboring zones of Gambella regional state (Zone 2) and the Southwest regional state (Kefisho Sheka) were selected purposively (Figure. 3.1). The selected zones cover the largest number of square kilometers of land, with abundant indigenous knowledge and natural products. The Zones of Southwest Ethiopia are characterized by highlands, lowlands, rugged areas, river valleys, and top-flat plateau lands and most of the zones are covered with forests of different vegetation. Among these, the Yayo Forest biodiversity reserve has been registered with UNESCO. The main rainy season is in summer months of June, July, August, and September (6–8).

The TM regulatory framework and legal basis assessment were conducted in three purposively selected regions, namely Oromia regional state, Addis Ababa city, and Southern Nations, Nationalities, and Peoples' (SNNPR) regional states. Out of 11 regional states, two were excluded from the study since they are newly established regions. The study consisted of federal medicine regulatory agencies as well as zonal, town or district level health regulatory offices in selected regions. In addition, the TMPs who are practicing in the respective regions were included in the study.

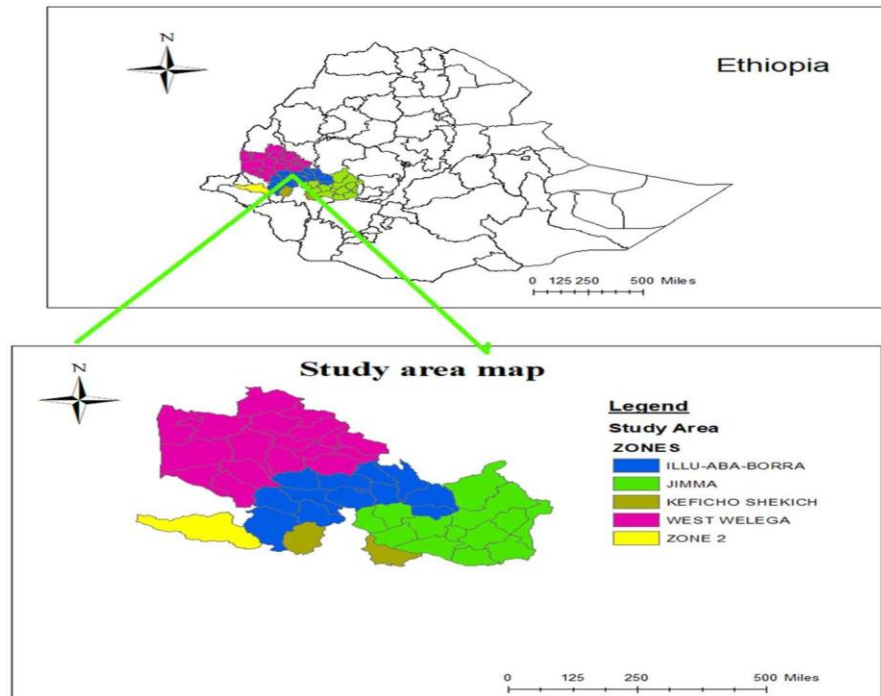


Figure 3.1: Authentic study area map with Geographic coordinates

### 3.4. Ethnomedicinal Survey and Regulatory Framework Assessment

#### 3.4.1. Population

##### Source Population

The source population for the study will all Traditional healres available in southwest Ethiopia regions(SW Oromia, Gambella and and Soethwest Ethiopia Zones),

##### Study Population

The study population for the will be all Traditional healres potentially available in selected southwest Ethiopia (Ilubabor, Bono Bedele, Shakicho and Majang(Zone 2) of Gambella)) .

##### Study Unit

All Traditional healers who fulfilled the inclusion criteria in respective study areas and periods.

## **Inclusion and Exclusion Criteria**

### **Criteria**

The study included in the study will be Traditional healers aged 18 years or older, who were willing to divulge information.

### **Exclusion Criteria**

Traditional healers who were not able to communicate, will be excluded from study

### **3.4.2. Sample Size Determination and Sampling Techniques**

The investigator approached the Traditional healers (key informants) through community leaders using the unstructured snowball method. There was no sampling method used for the selection of KI. As mentioned above, three zones of Western Oromia regional state and two neighboring zones of the Gambella regional state and the Southwest regional state were selected purposefully for ethnomedicinal purposes, and all available TMPs who fulfilled the criteria were included in the ethnomedicinal survey. For regulatory framework assessment, all federal-level TM regulatory authorities (EFDA) and MoH fulfilled the inclusion criteria purposively included in the study. But for regional states and city administrations, using the rule of thumb (9–11) 30% of zonal and districts (weredas) were selected randomly. Hence, from Oromia regional states, 7 zones and 9 districts were selected; from SNNP regional state, 5 zones and 5 districts were selected; and from Addis Ababa City, 4 sub-cities and 14 local city administrations were selected. From all respective regional states, 15 key informants from registered TMP who fulfilled inclusion criteria were included up to saturation of information. Finally, from federal level regulatory authority 57 participants, from Oromia regional state, 58 participants, from SNNP regional state, 41 participants, and from Addis Ababa city, 81 participants, were interviewed. Therefore, 57 federal-level officers, 180 regional-level regulatory officers, and 15 TMP were included in the study.

### **3.4.3. Data Collection Instrument and Procedure**

The Traditional Medicinal Practitioners (KIs) were interviewed using a semi-structured interview protocol. Data on indigenous knowledge about the perception and explanation of various infectious diseases, methods of diagnosis, medicinal plants used for the treatment of common infections, plant parts used, plant growth habits, and preparation approaches were collected. Additionally, the data collectors observed some treatment practices and the plants used by TMPs. At the end of the interview, the medicinal plants were collected from natural flora or home gardens with the help of the TMP. The collected plant samples were dried, authenticated, and deposited at Mattu University's Biology Department herbarium. Identification was performed using taxonomic keys and flora by comparison with the already identified herbarium specimens by the botanist of the department. The botanical names, along with their authority citations and plant family names, were checked with: [www.tpl.org](http://www.tpl.org), [www.eflora.com](http://www.eflora.com), and [www.ipni.org](http://www.ipni.org). Ethiopia's regulatory framework assessment data was collected using a combination of an archive assessment of Ethiopia's legislative basis, in-depth interviews, self-administrative questionnaires', and literature searches. Each data collection technique was primarily based on the WHO national TM policy guidelines and other related publications (12–14). The questionnaires included socio-demographic information, regulatory personnel's awareness and attitude towards TM regulation, systems and functionality of TM regulatory activities, TM regulation plans, monitoring and evaluation parameters, challenges, and prospective opportunities related to the implementation of TM regulation practices. The review tool includes a full explanation of the general content of the medication law as well as a checklist for the functions of the medicine regulatory authority. The comprehensiveness of the legal framework in Ethiopia was assessed through an archival evaluation. A trained BSc health professional, chemist, biologist, and folklorist collected all the data under the supervision of the principal investigator.

### **3.4.4. Validity and Reliability of Data Collection Tools**

The prepared interview guide was translated into Amharic and Afan Oromo and back into the English language to check its consistency. For all data collection tools, a pre-test was conducted on 5% of sample size, to test the interview guide other than data collection sites (West Wolega Zone and Sidama regional state). Based on the pre-test results, the interview protocol was re-corrected and administered to the participants. Traditional Medicine

regulation assessment tools were thoroughly checked and revised against the legislative framework of TM regulations and their practical implementations. All data collection tools were cross-checked and validated.

#### **3.4.5. Criteria for Plant Material Selection**

Based on the information obtained from ethnomedicinal survey, plant species were selected for antimicrobial activity and phytochemical screening by setting criteria (Table 3.1). These criteria include the easy accessibility of the plants, traditional claims, and some literature evidences (15–18). Based on these criteria, twelve medicinal plants were selected and were collected from Ilu Aba Bor Zone Forest, Oromia, southwest Ethiopia (34° 52' 30" E to 36° 5' 30" E longitudes and 7° 27' 30" N to 8° 49' 30" N latitude). *Justicia schimperiana* (Hochst. ex Nees), *Croton macrostachyus* Hochst. Ex Delile, *Albizia gumifera* (J.F.Gmel.) C. A. Sm., *Clematis hirsuta* Guill, and Perr, *Solanum nigrum* L., *Dodonaea angustifolia* L.f., *Crinum abyssinicum* Hochst. ex A. Rich, *Trichilia dregae* Sand, *Dracaena steudneri* Engl. Root, *Momordica foetida* Schumacher et Thonn, *Thalictrum rhynchocarpum* Quart.-Dill. & A.Rich, *Pycnostachys abyssinica* Fresen, were selected for antimicrobial test and qualitative phytochemical screening. Chloroform fraction of *C. abyssinicum* Hochst. ex A. Rich, *C.hirsuta* Guill. and Perr and *T.hynchocarpum* Quart.-Dill. & A.Rich) exhibited the lowest MIC, and hence selected for antimicrobial modulating effect evaluation. Among the above selected plant species, *C. abyssinicum* Hochst. ex A. Rich was selected for compound isolation since it showed, remarkable separation in addition with better antimicrobial efficacy.

Table 3.1: Criteria for selection of plant species to conduct ant-microbial test and phytochemical screening (Table 3.1)

| S.N | Listed plant species                                  | Local name         | Plant part | Traditional claim for 3 or more diseases | FI/% | No of Citation | Color change on phytochemical screening | TLC spots > 3 | TLC separation | Evidence of bioactivity test | Evidence of compound isolation | Plant selected for antimicrobial test | Plant selected for compound isolation |
|-----|-------------------------------------------------------|--------------------|------------|------------------------------------------|------|----------------|-----------------------------------------|---------------|----------------|------------------------------|--------------------------------|---------------------------------------|---------------------------------------|
| 1   | <i>Justicia schimperiana</i> (Hochst. ex Nees)        | Dhumuga a/sensel   | Root       | Yes                                      | 32   | 3              | Medium                                  | Yes           | P              | No                           | No                             | Yes                                   | No                                    |
| 2   | <i>Croton macrostachyus</i> Hochst. Ex Delile         | Bakaniisa a/bisana | Stem bark  | Yes                                      | 69   | 6              | High                                    | Yes           | P              | No                           | No                             | Yes                                   | No                                    |
| 3   | <i>Albizia gumifera</i> (J.F.Gmel.) C. A. Sm          | Ambabesa           | Stem bark  | Yes                                      | 24   | 3              | Medium                                  | Yes           | Me             | No                           | No                             | Yes                                   | No                                    |
| 4   | <i>Clematis hirsuta</i> Guill. and Perr               | YeAzo-hareg        | Whole part | Yes                                      | 31.7 | 2              | High                                    | Yes           | P              | No                           | No                             | Yes                                   | no                                    |
| 5   | <i>Solanum nigrum</i> L                               | Iddii adii         | Fruit      | Yes                                      | 72   | -              | Very high                               | Yes           | P              | No                           | No                             | Yes                                   | no                                    |
| 6   | <i>Dodonaea angustifolia</i> L.f.                     | Kitkita/Ittacha    | Leaf       | Yes                                      | -    | 3              | Medium                                  | Yes           | P              | No                           | No                             | Yes                                   | No                                    |
| 7   | <i>Crinum abyssanicum</i> Hochst. ex A. Rich          | Qulubi warabessaa  | Root bulb  | Yes                                      | 39   | 1              | Very high                               | Yes           | H              | No                           | No                             | Yes                                   | Yes                                   |
| 8   | <i>Trichilia dregae</i> Sand                          | Luuyaa             | Stem bark  | Yes                                      | -    | 1              | High                                    | Yes           | P              | No                           | No                             | Yes                                   | No                                    |
| 9   | <i>Dracaena steudneri</i> Engl. root                  | Sarxee             | Root       | Yes                                      | 40   | 1              | Medium                                  | No            | P              | No                           | No                             | Yes                                   | No                                    |
| 10  | <i>Momordica foetida</i> Schumach. et Thonn           | Saramba'oo         | Leaf       | Yes                                      | 3.0  | 1              | Poor                                    | No            | P              | No                           | No                             | Yes                                   | No                                    |
| 11  | <i>Thalictrum rhynchocarpum</i> Quart.-Dill. & A.Rich | Siraabizuu         | Root       | Yes                                      | 31.7 | -              | Medium                                  | Yes           | Me             | No                           | No                             | Yes                                   | No                                    |
| 12  | <i>Pycnostachys abyssinica</i> Fresen                 | Yeeroo             | Root       | Yes                                      | 25   | 1              | Medium                                  | No            | P              | No                           | No                             | Yes                                   | No                                    |

Key:- H: high, Me: Medium, P; poor



### **3.5. Materials, Chemical, and Microbial Strains, Equipments and Apparatuses**

Beakers, conical flask, measuring cylinders (different size), glass funnels, glass stirrer, cotton wool, spatula, Bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holders, refrigerator, meter rule, bottles, cabinet tripod stand, wire gauze, capillary tubes, filter paper, autoclave(NEUPS12 50L), centrifuge (NEUHC22), UV box with UV lamp(Quartz Lamp 110V 36W), TLC paper, column chromatography different sizes(Thermofisher), 500 MHz SB Bruker Avance NMR Spectrometer, LC-MS machines (Thermo LTQ Orbitrap) were some material used for extraction fractionation and isolation of pure compound

#### **Chemicals and reagents**

Analytical standards of chloroform, methanol, n-hexane ethyl acetate (Lneos Solvents Belgium), ferric chloride, HCl, Mayer-Wagner reagent (2.5 gm of iodine is dissolved in 12.5 gm of KI<sub>2</sub> with 250 ml of distilled water) , magnesium ribbon , NaOH , sulfuric acid, potassium ferricyanide (K<sub>2</sub>Fe (CN)<sub>6</sub>), dimethyl sulfoxide (DMSO), (Mettler-Toledo India Pvt.Ltd), Mueller Hinton Broth (Thermo Scientific<sup>TM</sup> ), Gentamycin, Dicloxacillin, Ciprofloxacin, Amphotericin B (Bactigen FDC Limited), and clotrimazole (Glenmark Pharmaceuticals Ltd). Were some chemicals, reagents and reference drugs used for this study.

#### **Test organism**

*Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC43300), *Escherichia coli* (ATCC 25922), *Salmonella enterics* NR13555 *Salmonella typhi* cbc, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Shigella flexneri*, *Leishmania donovani*, and *Candida albicans* (ATCC 90028) American type strains were kindly donated by EPHI and Yaoundé I University biomedicine department.

### **3.6. Extraction and fractionation of plant materials**

The air-dried and pulverized plant materials were extracted by maceration by dissolving it in chloroform/methanol 1:1 (v/v) for three consecutive days for 24 hours each. Some literatures have recommended that chloroform can penetrate plant cell walls and enhanced the extraction of highest lipid yield, we used the chloroform/methanol combination for extraction (19–21). The extracts

were concentrated using a rotary evaporator at a temperature of 40 °C to obtain crude extracts, which were subjected to phytochemical screening and antimicrobial evaluation. The crude extracts were again suspended in water and further partitioned using n-hexane, chloroform, and methanol. Each fraction of the plant extracts was then concentrated using rotary evaporator; scanted and dried by putting it in warm mantle using desiccator to remove the solvent residue based on previous studies (22–25)



Figure 3.1: Some pictures of extraction by maceration

### 3.7. Phytochemical Analysis

#### 3.7.1) Qualitative tests

Preliminary qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, steroids, and terpenoids) present in the extracts following standard protocols (24–26).

**Test for tannins:** About 200 mg of the plant extract was boiled with 10 ml of distilled water; and 0.1% ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

**Test for alkaloids:** The plant extract was dissolved in 100 ml of water, filtered, and cooked in steam with 2 ml of the filtrate and three drops of 1% HCl. Then, 1 ml of the heated mixture was combined with 6 ml of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

**Test for saponins:** About 0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam indicated the presence of saponins.

**Test for flavonoids and glycosides:** 200 mg of the plant extract was mixed with 10 ml of ethanol and filtrated. Two ml of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 ml of distilled water and NaOH to 0.5 ml of crude extract, the formation of a yellowish color indicated

the presence of glycosides.

**Test for steroids:** About 1 ml of the crude extract was combined with 10 ml of chloroform and 10 ml of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

**Test for terpenoids:** The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 ml of crude extract with 2 ml of chloroform and 3 ml of sulfuric acid.

**Test for phenols:** About 1 ml of the extract was combined with three drops of  $\text{FeCl}_3$ , and 1 ml of  $\text{K}_2\text{Fe}(\text{CN}_6)$ . The formation of greenish-blue forms confirmed the presence of phenols.



Figure 3.2: Some picture of preliminary phytochemical test color change medicinal plants

**3.7.1. Thin layer chromatography (TLC):** Thin layer chromatography was performed on TLCplate (Alumnium silica gel pre-coated with layer thickness of 0.2 mm) using hexane/ethyl acetate mixtures (8:2) as an eluent. Spots were applied using capillary tube 1.5 cm from the bottom marked by a line ruled using a pin. The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which was covered immediately. When the solvent reaches the top of the plate lined by pensil, the plate was removed, marked and dried. The number of thespots was detected under UV at 254 and 366 nm wavelengths and spraying with spotting reagent,using iodine vapor (24,25,27).

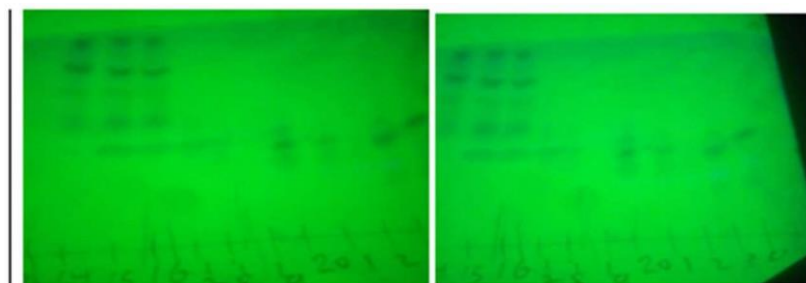


Figure 3.3: Some picture of TLC separation spots of selected medicinal plants.

### 3.8. Isolation of Pure Compounds

Based on the criteria stated under, table 3.1, the chopped and air-dried *C. abyssinicum* Hochst. ex A. was extracted and subjected to column chromatography. The number of spots was detected using TLC under UV at 254 and 366 nm wavelengths and by spraying with a spotting reagent using iodine vapor (28,29). For chromatographic separation, 5 cm diameter by 60 cm height column tubes were used; silica gel measuring 80 g (60–120 mesh) was used as the stationary phase, and 25 g (230–400 mesh) was used to adsorb the extract. From 70 g of air-dried plant powder 50 g (7.14%) of crude extracts were harvested. The crude extracts were then suspended in water and further partitioned successively with n-hexane, chloroform, and methanol.

The chloroform fraction which exhibited the lowest MIC was subjected to CC purification. Fractionation and isolation were carried out using column chromatography (Thermo Fisher Scientific GmbH Dreieich, Germany) over silica gel, gel filtration, and Sephadex LH-20 (Interra Global). Using hexane/ethyl acetate mixtures as an eluent, TLC was used to measure the degree of separation on plates of pre-coated aluminium silica gel with a layer thickness of 0.2 mm (Altmann Analytik GmbH & Co. KG, Germany). In a sequentially increased solvent polarity system, 135 fractions were obtained from n-hexane 100% up to ethyl acetate at 95% and methanol at 5%. Ca 10(1) compounds were obtained directly from fractions 5 to 13 after combining and washing (treating) them multiple times with the polar solvent methanol. 12 mg (0.05%) of a whitish powder with an R<sub>f</sub> value of 0.7 was harvested in a 70% n-hexane and 30% ethyl acetate solvent system.

The left fractions were reorganized according to their TLC profiles. All fractions were combined into four groups: fraction one (25–41), fraction two (42–69), fraction three (70–83), and fraction four (> 84f). Because TLC revealed the existence of numerous spots, all of the combined fractions were again subjected to CC. In the 5% n-hexane and 95% ethyl acetate solvent solution, fraction 25–41 produces 9 mg (0.04%) of brown powder compounds (Ca 36(2) isolated with an R<sub>f</sub> value of 0.52. The third compound (Ca 75) was isolated with an R<sub>f</sub> value of 0.45 after repeated eluting with 6 mg (0.03%) of white powder in a 20% n-hexane and 80% ethyl acetate solvent solution.



Figure 3.3: Pictures of plant material fresh (A), dried root bulb (B), powder (C) processing of CC (D), different eluted fractions and isolated pure compound

### 3.9. Structural Elucidation

The three isolated compounds sent to Bielefeld University, OC<sub>3</sub> research laboratory. The NMR spectra were recorded using a Bruker DRX-400 instrument (500 MHz SB BrukerAvance NMR Spectrometer), and the to avoid the overlapping of the large solvent peaks with the signal of the analytes deuterated solvents chloroform ( for compound 1) and acetone for (both compounds 2 and 3) were used. The sample dissolved in denatured solvent were placed in a magnetic field, then excite the nuclei sample into nuclear magnetic resonance with the help of radio waves to produce NMR signals, these NMR signals were detected with sensitive radio receivers and the NMR spectra were interpreted by correlating with standards and using chem-drown software. The NMR spectras of all compounds are listed under Annex 2.

The extraction, fractionation, antimicrobial tests, and the isolation of pure compounds were carried out at the department of chemistry, University of Yaoundé I, Cameroon, during the principal investigator's research stay, and at the Laboratory of Drug Quality (JuLaDQ), Jimma University. The NMR study was carried out at Germany's Bielefeld University OC<sub>3</sub> Research Group Laboratory. All of the experiments were conducted based on previous similar studies(30–32).

### 3.10. Antimicrobial Activity Tests

*In-vitro* MIC values of the extracts and fractions were determined using broth micro dilution method. Eppendorf tube was filled with 1gm of samples including the crude extracts and fractions of each plant. One ml of dimethyl sulfoxide (DMSO) was added to each tube containing plant extracts. The samples were vortexed in a geometric progression from 1000 µg/ml up to final dilution of 0.24 µg/ml. In all tubes, 100 µL of sterile Mueller Hinton Broth (MHB) culture was introduced. The microbial strain ( $2 \times 10^8$  CFU/mL) was inoculated into MHB liquid culture medium. Reference antibacterial (gentamycin, ciprofloxacin and dicloxacillin); anti-fungal (clotrimazole) and antileishmanial (amphotericin B) drugs were used as a positive control. Negative controls consist of the tubes containing only the culture medium on the one hand, and the tubes containing a mixture of broth culture and bacteria or the fungus on the other hand. After 24 hours of incubation at 37°C, the turbidity was observed as an indication of growth. All tests were performed in triplicate to confirm the activity. The minimum inhibitory concentration (MIC), which is defined to be the lowest concentration of the sample that prevents the growth of bacteria, was calculated.

An anti-promastigote assay was performed to determine the *in vitro* minimum inhibitory concentration (MIC) on an already cultured *L. donovani* strain. In a 96-well microplate assay, appropriately diluted extracts were added to the Leishmania promastigotes culture ( $2 \times 10^6$  cells/mL). The plate was incubated at 26 °C for 72 h, and the growth of Leishmania promastigotes was determined. Crude extract, the fractions and isolated compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with a liquid medium to final concentrations ranging from 100 to 1.5 µg/ml, maintaining the final DMSO concentration at a maximum of 0.05% w/v. All tests were determined based on previous work with slight modifications(6,7).

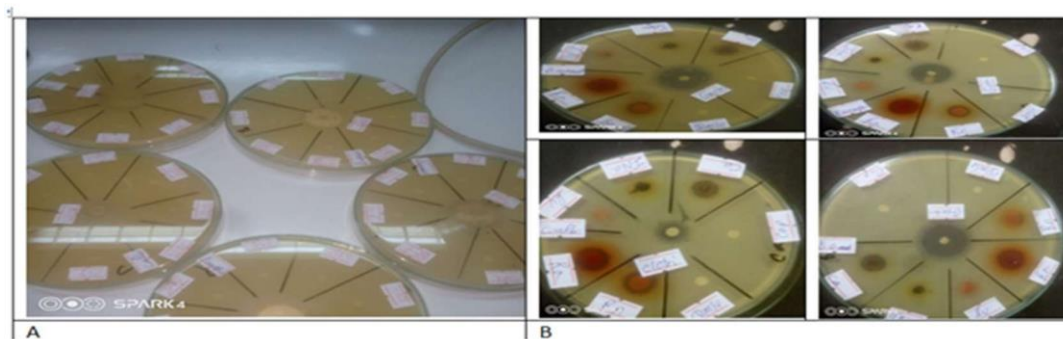


Figure 3.4 pictures of disc-diffusion of antimicrobial activity test of during before incubation and (A) and after incubation of the plant extract with inoculated microbial

### 3.11. Antibiotic Modulating Effect Assay

The combinational or interactive inhibition was determined using the checkerboard method for measurement of the synergistic antibiotic-modulating effects of plant extract based on previous studies (8,33–35). The checkerboard method is often combined with the calculation of a fractional inhibitory concentration (FIC) index to test the antimicrobial efficacy of drugs in combination (two anti-microbial are tested in double serial dilutions, and the concentration of each drug is tested both alone and in combination).

The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotics (Tetracycline, Amoxicillin, and Cloxacillin) with tree plant extracts having lowest MIC (*C. abyssinicum* Hochst. ex A. Rich chloroform fraction with MIC of 7.8 against *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 43300), *Clematis hirsuta* Guill. and Perr with MIC of 7.8 against *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 43300), and *Thalictrum rhynchocarpum* Quart.-Dill. & A.Rich Crude extract with MIC of 0.98 against both above selected bacterial strains. The above three conventional antibiotics were simply selected based on easily accessible in the lab and lowest zone of inhibition against selected two bacterial strains after conducting disc diffusion test. The FIC value for each agent was calculated using the formula  $FIC = MIC_{\text{Antibiotic in combination}} / MIC_{\text{Antibiotic alone}}$ , and the FIC index is interpreted as  $FIC < 0.5$  synergistic effect,  $0.5 < FIC < 1$  additive effect,  $1 < FIC < 4$  indifferent effect, and  $FIC > 4.0$  antagonistic effect(34,35).

For the disc diffusion test, serial nutrient agar plates were prepared by pouring molten media into sterile Petri dishes. The inoculums were then swabbed uniformly and allowed to dry for 5 minutes. Then, the filter paper discs (Whatman No. 1) measuring 6 mm were impregnated with the test samples and placed on the surface of the medium. The Petri dishes were incubated at 37 °C for 24 h, and the diameter of the zone of inhibition was measured in mm. A similar procedure was adopted for the positive control antibiotics mentioned, and the corresponding zone of inhibition was compared accordingly. One percent of DMSO was used as a negative control, and the inhibition zones are divided into three  $\leq 10$  mm diameter is categorized as weak or resistant, 11–20 mm is active, and  $\geq 21$  mm is very strongly active based on previous similar studies with slight modification (36–38).

### **3.12. Data Processing and Analysis**

All collected information, notes taken, and audio recordings were transcribed and translated into English. The organized data was entered into Epi-Data Manager Version 4.6. Then it was exported and analyzed using IBM® Statistical Package for the Social Sciences (SPSS) version 25.0, USA, and summarized using simple descriptive statistics. The frequency index (FI) corresponds to the percentage of informants that mention the use of the plant species for the management of ailments, given by  $FI = FC \times 100 / N$ , where FC denotes the number of informants who mentioned the use of the plant species to treat various infections, and N denotes the total number of informant (39,40). Bivariate and multivariate analyses were used to identify factors associated with the weak performance of TM regulatory offices in the implementation of TM regulation. Non-parametric one-way ANOVA analysis, Kruskal-Wallis and Dunn's tests were used for comparison of the overall association of the plant species as well as fractions of the extracts on MIC values of (41,42)). Statistical significance was defined at a level of 0.05, and the data was described with a confidence interval of 95%.

### **3.13. Data Quality Control**

Standard semi-structured questionnaires were prepared and translated to Amharic, Afaan Oromo and back to English to check consistency. A pre-test was conducted to 5% of the sample size at East Wolega zone for ethnomedicinal survey and at Sidama regional state for TM regulatory frame work analysis. Based on the results of the pilot study, questionnaires were re-corrected and administered to the participants. Training protocol was developed and training was given for data collectors and supervisors for two days (16 hr.). Each informant was supervised in order to confirm the reliability of the information. The responses that were not in harmony with each other were rejected. Plant material collection, storage, and transportation were kept clean. An attempt was made to prevent any contamination, and the investigators strictly followed standard procedures for all experimental analyses. Each experiment was conducted in triplicate. The equipment, culture media, and microbial strains used for the test were obtained from qualified companies, and all materials and equipment were checked for quality and calibration. The entire tests were based on previous studies with little modification (23,24,43).

### **3.14. Ethical Considerations**

The ethical clearance was obtained from the Institutional Review Board (IRB) of Jimma University Health Institute with the letter reference number JHRPG/720/2020. Oral consent was



obtained from the participants after an explanation of the aims and purposes of the study, their confidentiality and privacy were assured.

### **3.15. Operational Definitions of Some Terminologies**

**Ethnomedicine:** A study or comparison of the traditional medicine practiced by various ethnic groups, and especially by indigenous peoples.

**Herbal medicine:** Include herbs, herbal materials, herbal preparations and finished herbal products that contain, as active ingredients, parts of plants, other plant materials or combinations thereof.

**Herbalist:** TM practitioner who deliver TM service by using herbal medicine.

**Drug “repurposing”:** Using an existing drug for a new treatment that was not indicated before.

**Drug “reusing” or “restoring”:** combination the drug to enhance its action and returning its action to the same indication

### **3.16. Dissemination of the Results**

Parts of the study findings were communicated to the scientific community through publication in reputable peer-reviewed journals and also presented at national conferences. The findings will be also communicated to relevant bodies, including Jimma University, FMOH TM Desk, Traditional and Modern Medicine Research Institution, EFDA, and other organizations, in the form of a written document, a seminar, and a soft copy.

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# 4

## CHAPTER IV:

### ETHNOMEDICINAL PLANTS AND ASSOCIATED INDIGENOUS KNOWLEDGE FOR THE TREATMENT OF DIFFERENT INFECTIOUS DISEASES IN ETHIOPIA

Parts of this chapter were published as

**Dubale, S.**, Abdissa, N., Kebebe, D., Debella, A., Zeynudin, A., & Suleman, S. (2023). Ethnomedicinal plants and associated indigenous knowledge for the treatment of different infectious diseases in Ethiopia. *Journal of Herbal Medicine*, 40(May), 100669. <https://doi.org/10.1016/j.hermed.2023.100669>

## **Abstract**

**Background:** The majority of Ethiopians depend on ethnomedicinal for their health care needs. However, indigenous knowledge of ethnomedicinal is usually not documented, transferred orally from generation to generation.

**Aims:** This study aims at collecting, documenting, and generating related evidence of the ethnomedicinal use of the plants in southwest Ethiopia.

**Materials and Methods:** An ethnomedicinal survey was conducted in different administrative zones of three regional states in southwest Ethiopia. In addition, a literature search was conducted on ethnomedicinal used for the treatment of perceived infectious diseases in Ethiopia.

**Results:** Seventy medicinal plant species belonging to 40 plant families were identified from the ethnomedicinal survey; from which 30 (44.9%) plant species were newly reported for their claimed use against respective infections in the study area. *Vernonia amygdalina* Delile, was the most frequently used plant species. Intestinal parasites 15(21.4%) were the most common infections for which these top-listed plants were traditionally used. Consumer trust in ethnomedicinal 87(53%) was the primary reason for community preference for ethnomedicinal practices.

**Conclusions:** In this study, *V. amygdalina* Delile was among the most commonly reported plant species. The communities use more ethnomedicinal than modern medicine, because they have more trust in it. The information generated from this study is significant in the documentation and prioritization of plant species for further investigation.

**Keywords:** Antimicrobial, primary health care, traditional medicine, traditional medicinal practitioners



#### **4.1. Introduction**

Infectious diseases are the major cause of morbidity and mortality, accounting for approximately half of all deaths in the tropics (1,2). Bacterial infections account for the majority of infections. Furthermore, the development of drug resistance in bacteria appears to be the leading cause of treatment failure (3). Traditional medicinal plants have been used to treat a variety of infections for centuries (4–6). In many regions of the world, TMs are either the primary source of health care or used as a supplement. But TM information, particularly on the African continent, has been passed down orally for centuries (7,8).

In Ethiopia, medicinal plants are said to be used by more than 70% of the human population and 90% of the animal population (2,9,10). But, ethnomedicinal information is often orally transmitted, potentially causing its disappearance over time. The country's medicinal plants face threatened due to different factors (10,11).

As a result, this study aims to gather and document ethnomedicinal information and medicinal plants used for the treatment of perceived infection in Ethiopia. The gathered data will be added up to the country's medicinal plant database. The work provided evidence for additional exploration and paved the way for the identification of low-cost and active antibacterial lead compounds.

#### **4.2. Methods and Materials**

##### **4.2.1. Study Design**

As mentioned under chapter III, the study involved a cross-sectional ethnomedicinal search and a comprehensive literature review to collect detailed information about medicinal plants used for different infectious diseases and associated indigenous knowledge in Ethiopia. The data included plant species, plant parts used, routes of administration, modes of preparation, and how the community designates different infections, as well as methods for gaining and transferring traditional knowledge.

##### **4. 2.2. Study Area and Period**

The ethnomedicinal survey was conducted in southwest Ethiopia from January to September 2020.

##### **4. 2.3. Data Collection Procedure**

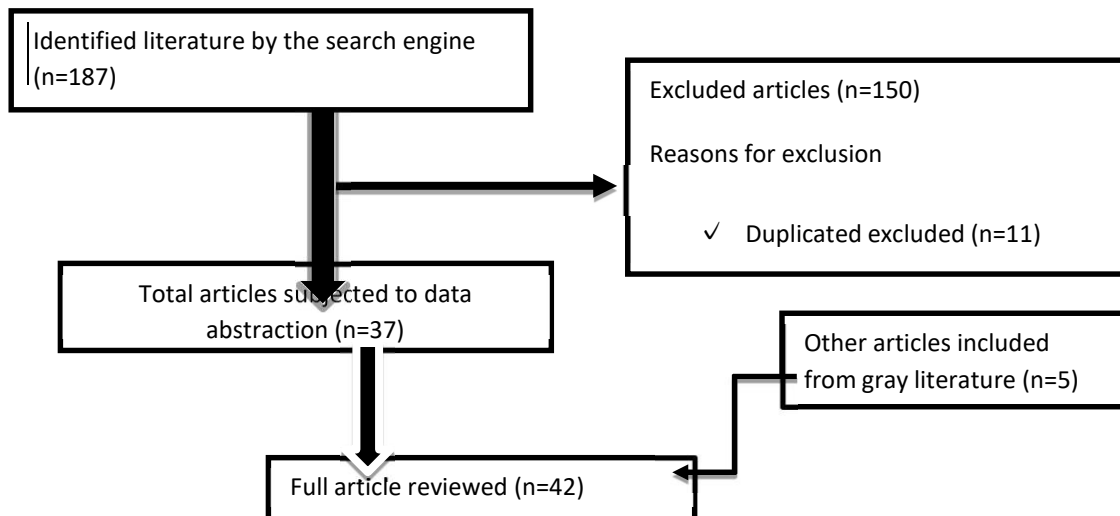
The data collection was conducted with the assistance of the community leaderes, local people,

public administrators, and field assistants. Initially, all TMPs were identified, and those who fulfilled the inclusion criteria were interviewed. The TMPs were interviewed using a semi-structured interview protocol (Chapter III, Section 3.3).

#### 4.2.4. A Literature Search Strategy

A literature search was conducted on medicinal plants that are traditionally claimed to be used for the treatment of perceived infectious diseases in Ethiopia using different search engines. Information obtained from the literature review was used to validate the traditional claims on the use of the medicinal plants for the treatment of infectious diseases. Two independent data collectors screened the articles based on the inclusion and exclusion criteria (Figure 4.1 and chapter III section 3.2).

The articles were selected according to the following steps:



**Figure 4.1:** Flow chart of data abstraction steps

### **4.3. Results**

#### **4.3.1. Socio-Demographic Characteristics of TMPs**

One hundred and sixty-four (164) TMPs were identified and all of them agreed to take part in the study. The study identified that most of the TMPs 122(74%) were male, live in a rural area (127/77%), and were farmers in their occupation 123(75%). The mean age of the TMPs was  $60 \pm 13$  years, the mean working experience as TMPs was  $42 \pm 15$  years, and the mean family size was  $5 \pm 2$  children (Table 4.1).

**Table 4.1: Socio-demographic characteristics of TPMs from January to September 2020 N=164**

| Variables                                                                 |                       | Frequencies/N | Percentage (%)    |
|---------------------------------------------------------------------------|-----------------------|---------------|-------------------|
| Gender of TPMs                                                            | Male                  | 122           | 74.00             |
|                                                                           | Female                | 42            | 26.00             |
| Residence                                                                 | Urban                 | 37            | 23.00             |
|                                                                           | Rural                 | 127           | 77.00             |
| Occupation (working status)                                               | Only as TMP           | 12            | 7.30              |
|                                                                           | Merchant              | 13            | 8.00              |
|                                                                           | Farmer                | 123           | 75.00             |
|                                                                           | Retired               | 16            | 10.00             |
|                                                                           | Gov,employ            | 9             | 5.00              |
|                                                                           | Housewife             | 21            | 13.00             |
|                                                                           | Religious leader      | 15            | 9.00              |
| Work as a TMP                                                             | Full time             | 12            | 7.00              |
|                                                                           | Parttime/as required  | 152           | 93.00             |
|                                                                           | Single                | 14            | 9.00              |
| Marital status                                                            | Married               | 126           | 77.00             |
|                                                                           | Widowed               | 14            | 9.00              |
|                                                                           | Divorced              | 10            | 6.00              |
|                                                                           | Cannot read and write | 79            | 48.00             |
| Educational status                                                        | Read-only             | 8             | 5.00              |
|                                                                           | Read and write        | 59            | 36.00             |
|                                                                           | Elementary school     | 12            | 7.00              |
|                                                                           | Secondary school      | 6             | 4.00              |
| Description of age, experience, family household income of size, and TPMs |                       |               |                   |
| Variables                                                                 | Minimum               | Maximum       | Mean $\pm$ (SD)   |
| Age of THs in year                                                        | 30.00                 | 90.00         | 64.00 $\pm$ 13.00 |
| How long the THs lived in the area                                        | 14.00                 | 88.00         | 57.80 $\pm$ 17.50 |
| Family size of THs                                                        | 1.00                  | 13.00         | 5.00 $\pm$ 2.21   |

#### 4.3.2. Indigenous Designations of Suspected Illnesses by Traditional Medicinal Practitioners

The study identified the indigenous designations given by TMPs. As presented in Table 4.2, some identified designations include *Dhukuba dhiiraa* (male disease), which refers to any disease affecting male sexual organs and caused by sexual contact. *Ija namaa* (Evil eye): evil-eyed people cause children to be affected by gastrointestinal illnesses. This is said to happen when an evil-eyed person looks at children while they are eating. The illness is manifested by crampy abdominal pain, nausea, vomiting, and diarrhea. *Michii*/sudden onset of respiratory tract illness: allergic eczema, allergic rhinitis etc caused by eating spicy foods outdoor and not washing hands afterward. *Ooqxoo* (severe itching all over the body): this can be caused by washing in selected river water (onchocerciasis). *Qufaa qorsaa* (severe cough that can affect the lungs): this can be caused by the inhalation of some vapor from chemically contaminated rain and bleaching from the sky, which can affect lung, manifested by the spitting of blood. *Rommisisaa* (shivering): having an acute high fever and abnormal shaking or trembling of body parts caused by the bad smell of stagnant water. *Fincooftu halkanii* (Night Bird Disease or Bat Urine Disease): the TPMs and some community members stated that bats urinate only once a year and contact with this urine causes inflammation and wounds (cutaneous leishmaniasis) on exposed human body parts. *Sabbata waaqayyoo* (Rainbow Disease): this explains chronic liver cirrhosis that can be interpreted by jaundice that is suspected to be due to urinating toward the rainbow direction. *Tibijii(O)*: necrotizing wound on the legs and on the back of the body, caused by the biting of an unknown insect. The additional native names of selected diseases, their correlated English meanings, descriptions, and perceived causes of these illnesses are listed in Table 4.2.

**Table 4.2 .TMPs and community members' indigenous naming, description, and perception of the causes of some suspected illnesses**

| <b>Local designations</b> | <b>Direct translation</b>                        | <b>English translation of related Illness</b> | <b>TMP and/or community description of the disease</b>                                                                                                                          | <b>Causes' perception</b>                             |
|---------------------------|--------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| Cittoo                    | Itching of the skin                              | Scabies,                                      | Mild itching, wounds over the skin                                                                                                                                              | Lack of washing of the body                           |
| Dhukuba dhiiraa           | Males disease                                    | Disease affecting sexual organ                | Any sexual organ-related diseases(e.g. gonorrhea)                                                                                                                               | Sexual contact                                        |
| Dhukuba saree             | Dog disease                                      | Rabies                                        | Mental disturbance caused by infection with mentally ill dog                                                                                                                    | Bitten with rabies infected dog                       |
| Gagabdoo                  | Seizure, Fainting                                |                                               | Sudden failure as a result of something falling from heaven, or something entering the nose by an evil spirit from a culturally protected area, or some light entering the eye. | Something supernatural or possessed by an evil spirit |
| Bineenssa                 | Snakebite                                        | Snake poisoning, GI infection in children     | Poisoning by a dangerous snake bite                                                                                                                                             | Poison from the snake bite                            |
| Ija namaa                 | The evil eye/the bad human eye                   |                                               | When someone looks at the children while they are eating food, then the children become sick, which is manifested by severe abdominal cramps, nausea, vomiting, and diarrhea.   | Evil spirit with an evil eye                          |
| Likiftii                  | Sudden mental illness related                    | Mental illness                                | Because of the contamination of some once-mental by evil spirits, the patient becomes out of reality.                                                                           |                                                       |
| Michii                    | Sudden onset of skin rash, any allergic reaction | RTI or allergic reaction                      | Getting out after eating (especially spicy foods) without handwashing; some mild evil spirit may affect the patient. Sever                                                      | a gentle evil spirit                                  |

**Table: 4.2. Continued**

|                             |                               |                          |                                                                                                                                   |                                                                                        |
|-----------------------------|-------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Ooqxxoo                     | Severe itching                | Onchocerciasis           | itching all over the body                                                                                                         | Using specific river water to wash                                                     |
| Qufaa qorsaa                | Severe cough                  | RTI, TB?                 | Severe coughing affects the lungs and can cause spitting of blood                                                                 | bleaching chemical that falls from the sky and is inhaled or touched by the individual |
| Raammoo garaa keessaa       | Intestinal worms              | Intestinal parasites     | The fullness of the intestine with the worms and caused by eating soil-contaminated food                                          | Eat soil, eat soil, eat soil, eat soil, eat soil, eat spicy food                       |
| Romisisaa                   | Shivering                     | AFI/Malaria              | Having a high fever and abnormal movement of body parts                                                                           | Bad smell from stagnant water                                                          |
| Dhukkuba Sabbata Waaqayyoo  | Rainbow disease               | Chronic liver cirrhosis  | They interpreted jaundice and yellowish discoloration of the body as well as body fluid as looking like the color of the rainbow. | Urinating by standing in the rainbow direction                                         |
| Shararitii                  | Spider disease                | Local fungal skin,       | The spider spits on the skin or the spider moves on the skin part                                                                 | Spider fluid discharge or spider leg                                                   |
| Simbroo halkanii/fincooftuu | Night bird/ bat urine disease | Cutaneous Leishmaniasis, | The bats urinate only once a year and urinate on the exposed parts of human bodies                                                | Bat urine                                                                              |
| Tibijii                     | Necrotizing wound             | Herpes Zoster            | Necrotizing wounds on the legs and around the back                                                                                | Biting of an unknown insect                                                            |

#### 4.3.3. Ethnomedicinal Plants used to Treat Infectious Diseases in the Study area

Seventy medicinal plant species that belong to 40 different plant families were identified by the current ethnomedicinal study. Asteraceae (6), Fabaceae (6), Euphorbiaceae (5), and Lamiaceae (5) were the most frequently represented plant families. *Vernonia amygdalina* Delile (Eebicha) (N = 70, FI = 43.3), *Croton macrostachyus* Hochst. Ex Delile (Bakkaniisa) (N = 69, FI = 42), and *Ocimum gratissimum* L (Damakase) (N = 66, FI = 40) were the most commonly reported plant species for the treatment of different infections in our study. In addition, wild pig meat (*Sus scrofa*) (N = 6, FI = 3.7) and Ethiopian Hedgehog meat (Xaddee inn afaan oromo and Jart in Amharic) (*Paraechinus aethiopicus*) (N = 7, FI = 4.3) were the most common wild animals used for the treatment of human infections (Table 4.3). Leaves and roots were the most frequently used plant parts; oral and dermal routes were the most frequently used routes of administration; shrubs and herbs, were the most commonly used plant growth habits during the study period (Figure. 4.3, 4.4, and 4.5).



Table 4.3: ethnomedicinal plants used for the treatment of perceived infectious diseases by TMPs: scientific name, family name, growth habits, parts used, routes of administration, and voucher number

| Family name    | Scientific name                           | Local name               | F  | FI   | Part used            | Growth | Preparation                                                                                                           | Treated disease                                                             | Routes of admin. | Voucher No. |
|----------------|-------------------------------------------|--------------------------|----|------|----------------------|--------|-----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|------------------|-------------|
| Acanthaceae    | <i>Justicia schipperiana</i> T. Anderson  | Dhummuugaa               | 32 | 19.5 | L, R                 | Sh     | Crushed and mixed with butter and hot water, it can be applied to the skin, wrapped around the head, or taken orally. | Skin infection, Onchocerciasis, Sudden mental illness (likift)              | Oral Dermal      | P01         |
| Alliaceae      | <i>Allium sativum</i> L. c. rinum         | Qullubbii                | 48 | 29.3 | Bulb                 | H      | Eaten with an empty stomach                                                                                           | Cough, RTI, sinusitis, laryngeal infection, tonsillitis, allergic rhinitis, | Oral             | P02         |
| Amaryllidaceae | <i>C. abyssinicum</i> Hochst              | Qullubbii<br>Waraabessaa | 39 | 23.9 | L, root bulb, aerial | Sh     | applied to the affected area by mixing with water or given orally with honey.                                         | Cancer, skin infection, Leishmaniasis, rabies                               | dermal, or oral  | P03         |
| Anacardiaceae  | <i>Schinus molle</i> , L                  | Qundobarbaree            | 22 | 13.4 | Se                   | H      | Crushed and chewed in the larynx                                                                                      | Tonsillitis, laryngeal infection                                            | Oral             | P04         |
| Apiaceae       | <i>Centella asiatica</i> (L.)             | Buqee                    | 9  | 5.5  | Se, L                | Sh     | and rubbed or swallowed.                                                                                              | Abdominal pain, diarrhea, Tinea                                             | Oral Dermal      | P05         |
| Apidae         | <i>Apis mellipoda</i> stingless bee honey | Damma daamuu             | 13 | 7.9  | L                    |        | Given orally with coffee or alone,                                                                                    | STD, TB, COPD, GI infection                                                 | Oral, Dermal     | P06         |
| Apocynaceae    | <i>Carissa spinarum</i> L.                | Agamssa                  | 21 | 12.8 | Fe                   | Sh     | ripe fruit is eaten                                                                                                   | IP, GI infection, and evil eye                                              | Oral             | P07         |

Table 4.3: continued

| Family name  | Scientific name                                      | Local name           | F  | FI   | Part used | Growth | Preparation                                                                 | Treated disease                                                                                                                                        | Routes of admin. | Voucher No.  |
|--------------|------------------------------------------------------|----------------------|----|------|-----------|--------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|--------------|
| Asteraceae   | <i>Vernonia amygdalina</i> Delile                    | Eebicha              | 71 | 43.3 | L, SB     | Sh     | The extract is taken by mouth with honey.                                   | TB, RTI, gastritis, HIV, STD Crushed                                                                                                                   | Oral             | P08          |
|              | <i>Vernonia myriantha</i> Hook. f.                   | Reejiii              | 47 | 28.7 | L, SB     | Sh     | Leaf juice is given orally and applied to the skin.                         | Snake poisoning, rabies, wound infection, stop bleeding                                                                                                | Dermal, Oral     | P09          |
|              | <i>Bidens macroptera</i> (Sch.Bip. ex Chiov.) Mesfin | keelloo              | 15 | 9.1  | L         | H      | Not mentioned                                                               | Stop the bleeding. The antidote for red snake poison and IP, To expel the snake, the powder is smoked, or it is mixed with sweetener and taken orally. | Oral             | P010         |
|              | <i>Echinops kebericho</i> , Mesfin                   | Qabarichoo           | 28 | 17.1 | SB, R     | H      | Spoking                                                                     | Replanting of Cancroid snakes                                                                                                                          | Oral, Dermal     | P011         |
|              | <i>Cirsium englerianum</i> O. Hoffm.                 | Saramba'o/hu mbaa'oo | 29 | 17.7 | Se        | Sh     | Fresh leaf juice is squeezed and applied to a fresh wound or affected area. | Blood clotting, an antidote to snake poisoning                                                                                                         | Dermal           | P012         |
|              | <i>Acmella caulirhiza</i> Del                        | Guutichaa            | 5  | 3.0  | L Fl      | Sh     | Fresh flowers are chewed and swallowed.                                     | Tonsillitis, tooth                                                                                                                                     | Oral             | P013         |
| Boraginaceae | <i>Stereospermum kunthianum</i> Cham                 | Botoroo              | 16 | 9.8  | SB        | T      | Not stated                                                                  | ache, oral infection                                                                                                                                   | Oral             | P014<br>P014 |
|              | <i>Cordia africana</i> Lam                           | Waddeessa            | 27 | 16.5 | R, Se     | T      | Not stated                                                                  | STDs, GI infections, tuberculosis, HIV crushed root, mixed with water, and swallowed to treat GI infection, IP, and eye disease                        | Oral             | P015         |
|              | <i>Cynoglossum lanceolatum</i> Forssk.               | Maxxannee.           | 5  | 3.0  | R         | Sh     | The extract is taken by mouth with honey.                                   | TB, RTI, gastritis, HIV, STD Crushed                                                                                                                   | Oral             | P016         |

Table 4.3 continued

| Family name   | Scientific name                         | Local name     | F  | FI   | Part used | Growt h | Preparation                                                                                                                       | Treated disease)                                                                                | Routes admin.      | of | Vouche r no |
|---------------|-----------------------------------------|----------------|----|------|-----------|---------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------|----|-------------|
| Brassicaceae  | <i>Coronopus didymus</i> (L.) Sm        | Surmma Leencaa | 37 | 22.6 | L, R      | Sh      | Crushed and boiled fluid was given orally with different soups                                                                    | Bone fracture, infection,                                                                       | Oral, Dermal       |    | P017        |
|               | <i>Lepidium sativum</i> L               | Shinfaa        | 13 | 8.0  | Se        | H       | rousted, powdered, and given orally.                                                                                              | sinusitis, liver                                                                                | Oral               |    | P018        |
| Campanulaceae | <i>Canarina eminii</i> Asch. & Schweinf | Maracaa        | 36 | 22.0 | L         | Sh      | Crushed fresh leaves are given orally and applied to the affected skin part.                                                      | cirrhosis, the antidote for snake venom, rabies, sudden mental illness, onchocerciasis, scabies | Oral, Dermal       |    | P019        |
| Caricaceae    | <i>Carica papaya</i> L.                 | Papaya         | 19 | 11.6 | Se        | Sh      | Oral administration of roasted seed powder                                                                                        | Hemorrhoids, IP, elephantiasis, Herpes Simplex, other viral skin infections,                    | Oral               |    | P020        |
| Dracaenaceae  | <i>Dracaena steudneri</i> Engl.         | Sarxxee        | 65 | 40   | L, WP, Fe | Sh      | Crushed, mixed with butter, and applied; powdered, mixed, and administered orally; boiled, and massaged onto swollen lymph nodes. | tuberculosis, swelling, HIV, lymphadenitis                                                      | Dermal             |    | P021        |
| Euphorbiaceae | <i>Ricinus communis</i> L.              | Qobboo         | 24 | 14.6 | L, Se, R  | Sh      | Leaf, seed, or root crushed and given orally with coffee, water, or milk or used as a sitting bath for hemorrhoids                | Rabies infection, snake poison, IP, hemorrhoids, constipation, STD,                             | Dermal, Oral, Anal |    | P023        |
|               | <i>Euphorbia tirucalli</i> L.           | Annanoo        | 5  | 3.0  | Leaf WP   | H       | A drop of milky latex applied to the affected skin                                                                                | Fungal skin infection,                                                                          | Dermal             |    | P024        |

Table 4.3 continued

| Family name | Scientific name                                                               | Local name  | F  | FI            | Part used        | Grow form | Preparation                                                                                                                                      | Treated disease                                                                                                 | Routes of admin.        | Voucher No. |
|-------------|-------------------------------------------------------------------------------|-------------|----|---------------|------------------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------|-------------|
|             | <i>A. gumifera</i> (J.F.Gmel.)<br><i>C. A. Sm,</i>                            | ambabesa    | 22 | RB &<br>SB L, | SB, root<br>seed | T         | Sequeeze and given with honey. Aply on the<br>skeen                                                                                              | Leishmaniasis anti-helminthic (tapeworm),<br>antifungal, anti trypanosomal, anti<br>Leishmaniasis               | Po derma                | P)14        |
|             | <i>Euphorbia candelabrum</i><br><i>var. candelabrum Ex</i><br><i>Delile</i>   | Bakkaniissa | 69 | 42.1          | L, SB            | T         | The crushed dried powder is mixed with<br>water and honey and taken orally. The leaf is<br>rubbed on the affected skin, and smoke is<br>inhaled. | AFI, fungal and bacterial skin infections,<br>STDs, insect replants, allergies, herpes<br>zoster, Leishmaniasis | Dermal, Oral &<br>Nasal | P025        |
|             |                                                                               | Adaamii     | 27 | 16.5          | L, latex,        | T         | The white fluid latex is applied to the affected<br>part.                                                                                        | Fungal skin infection, hepatitis,                                                                               | Dermal, Oral            | P026        |
|             | <i>Senna septemtrionalis</i><br>(Viv) Barneby and Irwin                       | Sabanakii   | 12 | 7,3           | L                | Sh        | Crushed fresh leaves are mixed with water<br>and taken by mouth.                                                                                 | Snakebite, liver disease                                                                                        | Oral                    | P027        |
| Fabaceae    | <i>Trigonella foenum-<br/>graecum</i> L. sprouted                             | Sunqoo      | 35 | 21.3          | Se               | H         | Powder mixed with water or sugar is given<br>orally early in the morning, and boiled                                                             | GI infection, gastritis, fever, and dysentery.                                                                  | Oral,                   | P028        |
|             | <i>Viana unaiculata</i> (L.)<br><i>Milletia ferruginea</i><br>(Hochst.) Walp. | Nenhoo      | 16 | 9.8           | Se               | Sh        |                                                                                                                                                  | IP( intestinal Parasites                                                                                        | Oral. Dermal            | P029        |
|             |                                                                               | Sootaloo    | 13 | 8.0           | SB, L, Se        | T         | seeds eaten on an empty stomach, fresh leaves<br>smashed and rubbed on the affected part.                                                        | Scabies, tungiasis, exto-parasites                                                                              | Oral Dermal             | P030        |
|             | <i>Calpurnia aurea</i> (Ait.)<br>Benth                                        | Ceekaa      | 46 | 28.0          | L, SB, R         | Sh        | and applied to the affected part.                                                                                                                | TB, dysentery<br><br>STD (syphilis), IP (eye infection),<br>elephantiasis,                                      | Oral Dermal             | P031        |

Table 4.3 continued

| Family name | Scientific name                           | Local name        | F  | FI   | Part used | Grow form | Preparation                                                                       | Treated disease                                                                         | Routes of admin.    | Voucher No. |
|-------------|-------------------------------------------|-------------------|----|------|-----------|-----------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------|-------------|
| Fabaceae    | <i>Erythrina brucei</i> Schweinf          | Waleenssuu        | 3  | 1.9  | L, SB     | T         | Not stated                                                                        | Amebic dysentery                                                                        | Oral                | P032        |
|             | <i>Pycnostachys abyssinica</i> Fresen     | Yeeroo            | 41 | 25   | L         | Sh        | rubbed on the skin, and stored on and around the nursing mother's bed             | RTI, skin infection, scabies, prevention of evil spirits after delivery.                | Dermal              | P033        |
| Lamiaceae   | <i>Plectranthus edulis</i> (Vatke) Agnew. | Dinicha Oromo (O) | 19 |      | L, R      | H         | powdered and given in a coffee cup.                                               | Onchocerciasis, epilepsy, Evil Eye Elephantiasis                                        | Oral                | P034        |
|             | <i>Ocimum basilicum</i> L.                | Besobilaa         | 4  | 1.6  | WP        | H         | Crushed and mixed with food                                                       | RTI                                                                                     | Oral                | P035        |
|             | <i>Clerdendrum myricoides</i> Hochst      | Maraasisaa        | 41 | 25   | L         | Sh        | The fresh leaf is used to wash the whole of the body and some are given by mouth. | Herpes simplex, Leishmaniasis, Onchocerciasis, gastrointestinal infection, N/V/Diarrhea | Dermal, Oral        | P036        |
|             | <i>Ocimum gratissimum</i> L.              | Damakasee         | 66 | 40.0 | L         | H         | The juice is inhaled through the nose or rubbed on the skin (RTI)                 | pneumonia, common cold, headache, sinusitis, allergic skin, airway constriction         | Oral, Dermal, Nasal | P037        |

Table 4.3 continued

| Family name    | Scientific name                                            | Local name | F  | FI   | Part used | Grow th | Preparation                                                                                    | Treated disease                                                               | Routes of admin' | Vouche r No |
|----------------|------------------------------------------------------------|------------|----|------|-----------|---------|------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------------|-------------|
| Linaceae       | <i>Linum usitatissimum L.</i>                              | Talbaa     | 33 | 20.1 | Se        | H       | Powder soaked in water overnight, the water extract mixed with honey or sugar and given orally | GI infection, constipation, STD(Gomorra), ear infection (Sebaceous expulsion) | Oral             | P038        |
| Menispermaceae | <i>Stephania abyssinica</i> (Quart.-Dill. & A.Rich.) Walp. | Kalalaa    | 16 | 9.8  | WP        | H       |                                                                                                | GI infection in children ascariasis), Cholera                                 | Oral             | P039        |
| Moraceae       | <i>Ficus vasta Forssk</i>                                  | Harbbuu    | 37 | 10.4 | Fe        | T       | The fruit is taken orally                                                                      | IP, GI infection                                                              | Oral             | P040        |
|                | <i>Morus alba L</i>                                        | Goraa adii | 3  | 1.9  | Fe        | Sh      | Fresh ripen fruit eaten                                                                        | IP                                                                            | Oral             | P041        |
| Moringaceae,   | <i>Moringa streptocarpa</i> Chiov.                         | Shifera    | 7  | 4.3  | L         | Sh      | Dry powder mixed with tea and given orally                                                     | Herpes zoster, HPN, head ache                                                 | Oral             | P042        |
| Myrsinaceae    | <i>Embelia schimperi</i> Vatke                             | Haanquu    | 9  | 5.5  | Fe        | T       | Fruit is eaten on an empty stomach                                                             | Hypertension, different infections, IP                                        | Oral             | P043        |
|                | <i>Maesa lanceolata</i> Forssk.                            | Abbayyii   | 5  |      | L, Se     | T       | Crushed, mixed with butter, and applied                                                        | Leg swelling /Elephantiasis                                                   | Derma            | P044        |

Table 43 continued

| Family name     | Scientific name                           | Local name                       | Frequency | FI   | Part used | Grow form | Preparation                                                                                              | Treated disease                                                                                 | Routes of administration | Voucher No. |
|-----------------|-------------------------------------------|----------------------------------|-----------|------|-----------|-----------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------|-------------|
| Myrtaceae       | <i>Psidium guajava</i> L.                 | Zeeyituunaa                      | 6         | 3.7  | L         | Sh        | Not stated                                                                                               | Skin infection                                                                                  | Dermal                   | P045        |
|                 | <i>Eucalyptus globules</i> labing         | Baargamoo                        | 29        | 17.7 | L, SB     | T         | leaf boiled and inhaled                                                                                  | Bronchoconstriction, sinusitis, and the common cold                                             | Nasal                    | P046        |
| Phytolaccaceae, | <i>Phytolacca dodecandra</i> , L 'Hert.   | Andoodee                         | 61        | 37.2 | L, se. R  | Sh        | Crushed fresh seed or root is applied to the skin or on the affected area, early in the morning.         | Shistosomiasis (UTI), rabies, scabies, onchocerciasis                                           | Dermal, Oral             | P047        |
| Poaceae,        | <i>Cynodon nlemfuensis</i> Vanderyst      | Coqorssa                         | 18        | 11.0 | WP        | Sh        | Chewing and spitting on the affected site                                                                | Herpes simplex                                                                                  | Dermal                   | P048        |
| Polygalaceae    | <i>Securidaca longipedunculata</i> Fresen | Tsebenayii                       | 23        | 14.0 | R         | Sh        | Crushed and mixed with H <sub>2</sub> O                                                                  | IP, hepatitis                                                                                   | Oral                     | P049        |
|                 | <i>Rumex abyssinicus</i> Jacq             | Dhangaggoo                       | 39        | 22.0 | L, R      | Sh        | The juice of the crushed root, mixed with tea and given early in the morning,                            | HPN, DM, gastroenteritis, onchocerciasis, lymphatic filariasis/elephantiasis.                   | Oral                     | P050        |
| Ranunculaceae   | <i>Thalictrum rhyndocarpum</i> Dill       | Siraabizuu                       | 52        | 31.7 | R         | H         | The root is crushed and the squeezed juice is taken by mouth or chewed. The squeezed juice is swallowed. |                                                                                                 | Oral                     | P051        |
|                 | <i>Nigella sativa</i> L.                  | Gur'aa/<br>Abasuuda<br>gurraacha | 21        | 12.8 | Se        | H         | soaked in the water, mixed with honey,<br><br>and given orally or inhaled nasally                        | STDs, malaria, GI infections,<br><br>diarrhea, IP, and mosquito repellent powder, Leishmaniasis | Oral,<br><br>Nasal       | P052        |

Table 4. 3 continued

| Family name   | Scientific name                             | Local name  | F  | FI   | Part used | Grow form | Preparation                                                                                                    | Treated disease                                                             | Routes of admin.     | Voucher No |
|---------------|---------------------------------------------|-------------|----|------|-----------|-----------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------|------------|
| Ranunculaceae | <i>Clematis longicauda</i> steud ex.A. rich | Idda fiitii | 7  | 4.3  | Wp        | Sh        | Fresh root and leaf chewed                                                                                     | abdominal infection, Leishmaniasis                                          | Oral                 | P053       |
| Rhamnaceae    | <i>Rhamnus prnoides</i> L'Hér.              | Geeshoo     | 42 | 25.6 | L, Se, R  | Sh        | Crushed leaf, soaked in malt, stored in a tight clay container for five days, filtered and administered orally | GI infection, hepatitis, IP, scabies Leishmaniasis                          | Oral, Dermal         | P054       |
| Rosaceae      | <i>Prunus africana</i> (Hook. f.)           | Hoomii      | 22 | 13.4 | SB, L,    | T         |                                                                                                                | STDs, lymphadenitis, GI infections, and protest swelling                    | Oral, Derma, Genital | P055       |
|               | <i>Hagenia abyssinica</i> (Brace) J.F.Gmel. | Heexoo      | 9  | 5.5  | WP        | Sh        | Crashed, mixed with water, and given orally                                                                    | IP                                                                          | Oral                 | P056       |
| Rubiaceae,    | <i>Coffee arabica</i>                       | Buna        | 20 | 12.2 | Se        | Sh        | roasted, powdered, and mixed with honey.                                                                       | Diarrhea, amoebic dysentery                                                 | Oral                 | P057       |
| Rutacea       | <i>Citrus aurantifolia</i>                  | Loomii      | 26 | 15.9 | Fe        | Sh        | squeezed, and the fluid part sucked or inhaled through the nose                                                | Common cold, rhinitis, motion sickness prevention, Nausea, and vomiting     | Oral, Nasal          | P058       |
|               | <i>Ruta chalepensis</i> L.                  | Ceredaama   | 43 | 26.2 | L         | H         | Fresh leaves boiled with coffee or milk and given orally                                                       | GI infection NVD, RTI, and the common cold soothing respiratory tract pain. | Oral,                | P059       |
|               | <i>Clausena anisata</i> (Wild.) Benth       | Ulmayyaa    | 9  | 5.5  | L, SB     | Sh        | Brush teeth with stems, and powdered leaves mixed with water, and give to the victim right away.               | Rabies and snake bite                                                       | Oral                 | P060       |



Table 4.3 continued

| Family name   | Scientific name                                   | Local name   | F  | FI   | Part used | Growth | Preparation                                                                | Treated disease)                                 | Routes of admin. | Voucher No. |
|---------------|---------------------------------------------------|--------------|----|------|-----------|--------|----------------------------------------------------------------------------|--------------------------------------------------|------------------|-------------|
| Solanaceae    | <i>Withania somnifera</i> L. Dunal                | Koddoo       | 45 | 27.4 | Wp        | H      | Fresh leaves are crushed and given orally by mixing with water.            | N/V/D, GI infection, abdominal cramps, IP, AFICC | Oral Nasal       | P061        |
|               | <i>Datura stramonium</i> L.                       | Asaangra     | 11 | 6.7  | WP        | H      | Crushed and applied to the affected area.                                  | Eye infection, warts, nose bleeding wrinkled     | Dermal, Ocular   | P062        |
|               | <i>Solanum nigrum</i> L.                          | Iddii adii   | 72 | 44   | Fe,L,R    |        | Crushed and applied to the affected area.                                  | Wounds, Leishmaniasis, RTIF                      | Derml            |             |
| Suidae        | <i>Sus scrofa</i>                                 | Pig Meat     | 6  | 3.7  | Meat      |        | seasoned food is eaten with salt                                           | TB, HIV. Leishmaniasis                           | Oral             | P063        |
| Tiliaceae     | <i>Camellia sinesis</i>                           | Baala shayee | 23 | 14.0 | L         | Sh     | Roasted leaf powder is mixed with water and taken orally without sugar.    | Asthma, COPD                                     | Oral             | P064        |
|               | <i>Grewia ferruginea</i> Hochst. ex A. Rich.      | Dhoqonuu     | 3  | 1.8  | SB        | Sh     | Fresh SB is being shampooed.                                               | Scalp infection, dandruff, psoriasis,            | Dermal           | P065        |
| Verbenaceae   | <i>Lippia adoensis</i> Hochst. ex Walp            | Kusaa'ee     | 23 | 14.0 | Wp        | H      | RTI, Gastritis: fresh leaves chewed or powder given with coffee or inhaled |                                                  | Oral, Nasal      | P066        |
| Zingiberaceae | <i>Zingiber officinale</i> Roscoe                 | Gijinbila    | 34 | 20.7 | R         | H      | Chewed and swallowed, or gurgled and inhaled into the nose,                | Tonsillitis, laryngitis, and RTI                 | Oral, Nasal      | P069        |
|               | <i>Aframomum corrorima</i> (A.Braun) P.C.M.Jansen | Ogj'oo       | 13 | 8    | L, R, Se  | H      | Dried roused powdered added to coffee or local traditional food(Marqaa)    | Common cold, Malaria. Shivering, RIT             | Oral             | P070        |

Key: -AFI: Acute Febrile Illness L: Leaf, F: Frequency, Fe: flower, N/V/D: nausea, vomiting, Diarrhea Se: Seed, Fi: fruit, FI Frequency Index, IP: Intestinal Parasite, GI: GastroIntestinal, SB Stem Bark, H: Herb, T: Tree, Sh: Shrub, STD: Sexual Transmitted Diseases, Whole Part

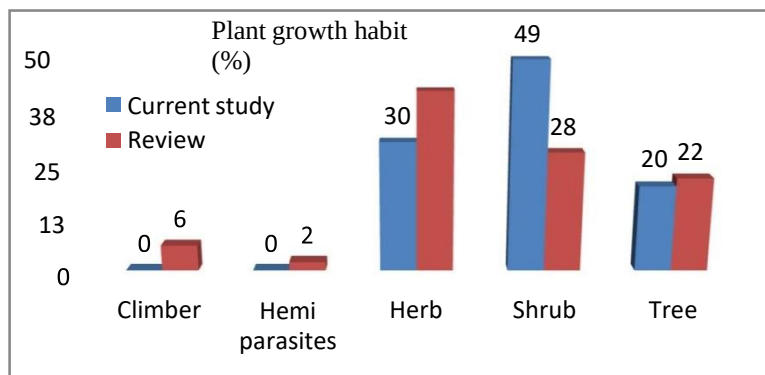


Figure 4.2: Summary of plant growth habit during the study period from the ethnomedicinal study and review results.

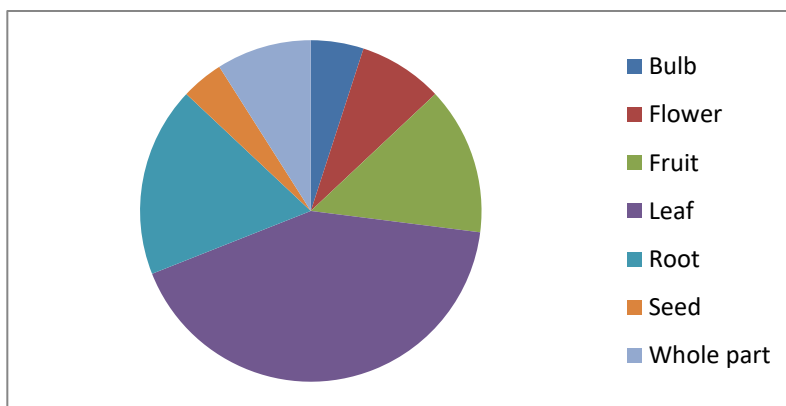


Figure 4.3: Summary of plant parts used during the study period from ethnomedicinal study and review results

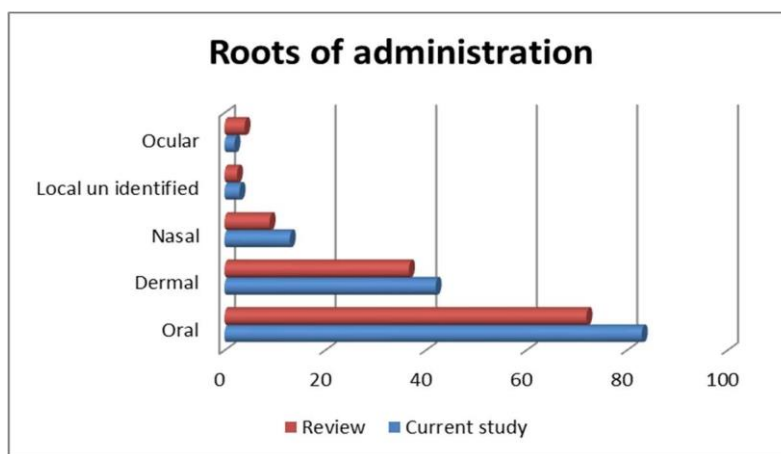


Figure 4.4: Summary of modes/roots of administration of the plants during the study period

#### **4.3.4. Ethnomedicinal Knowledge Transfer and Practices**

Traditional medicine practitioners were interviewed to generate information related to why communities prefer ethnomedicinal Knowledge services to modern medicine, how TMPs acquire the knowledge and practices, to whom they transfer their knowledge and practices, and the documentation of the services they provide. It was found that consumers' trust in ethnomedicinal 87(53%), the affordability of ethnomedicinal products??? compared to modern medicine 76(46%), and the ease of access to ethnomedicinal products 66(40%) were the primary reasons for community's preference for ethnomedicinal practices. Most of the TMPs 131(79%) learned from family members, and most of them 110(67%) were willing to share their knowledge. However, the vast majority of the respondents (80%) stated that they needed to pass on their knowledge verbally only to their favority children. One hundred and thirteen (69%) of TMPs responded that the younger generations are unwilling to practice traditional medicine, with the majority of them believing that ethnomedicine is an antiquated practice. Only a few TMPs (28%) stated that they were willing to reveal all the secrets of their practice, and collaborate with researchers to evaluate the safety and efficacy of their herbal medicines. The reasons given for their reluctance to disclose this secrete were: (1) belief that if the secret is revealed, the treatment will be ineffective 102(86%); and (2) being their main source of livelihood or income 46(39%) (Table 4.4).

Table 4.4: Information related to how TMP acquired and transferred the knowledge and documentation of the TM they practice during the study period in Ethiopia (N=164)

| Variables                                                                                                            | Responses                                                 | N   | %     |
|----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|-----|-------|
| The most important factors influencing the community's use of TM                                                     | Consumers have more confidence in TM than MM              | 87  | 53.00 |
|                                                                                                                      | It is cheaper than MM.                                    | 76  | 46.00 |
|                                                                                                                      | TM is easily available than MM                            | 66  | 40.00 |
|                                                                                                                      | TM is more effective than MM.                             | 14  | 9.00  |
| How did the TMP acquire knowledge of TM?                                                                             | From a close family member                                | 131 | 79.90 |
|                                                                                                                      | From a TMP while training                                 | 35  | 21.34 |
|                                                                                                                      | Learning from medicine books                              | 8   | 5.00  |
|                                                                                                                      | Learning from religious institutes                        | 3   | 2.00  |
| The response of the TMP about the transfer of their knowledge.                                                       | Yes                                                       | 110 | 67.1  |
|                                                                                                                      | No                                                        | 54  | 32.9  |
| To whom the TMP transfers their knowledge,                                                                           | To their children only                                    | 127 | 80.00 |
|                                                                                                                      | To relatives                                              | 22  | 13.00 |
|                                                                                                                      | To any person who needs it                                | 17  | 10.00 |
|                                                                                                                      | Not at all.                                               | 11  | 7.00  |
| TMP response about young generations' willingness to get information on TM                                           | Yes                                                       | 51  | 31.10 |
|                                                                                                                      | No                                                        | 113 | 69.00 |
| TMPs response about the reason why young generations do not have the willingness to get information about TM (N=113) | Thinking of TM as backward practice                       | 112 | 99.00 |
|                                                                                                                      | Thinking of TM as a harmful practice                      | 34  | 30.10 |
|                                                                                                                      | No reason                                                 | 14  | 11.00 |
| TMPs response about the documentation of TM knowledge                                                                | Yes                                                       | 20  | 12.00 |
|                                                                                                                      | No                                                        | 144 | 88.00 |
| If documented, in what way is the knowledge documented (N = 20)?                                                     | Published book, unpublished book/material                 | 7   | 35.00 |
|                                                                                                                      |                                                           | 17  | 85.00 |
| THs response about records of client's information (name, age, sex, illness, remedies given, etc.)                   | Yes                                                       | 8   | 5.00  |
|                                                                                                                      | No                                                        | 156 | 95.00 |
| TMPs response regarding remedies used to be documented and researched                                                | Yes                                                       | 56  | 34.00 |
|                                                                                                                      | No                                                        | 108 | 66.00 |
| Willingness to reveal the secret of TM knowledge and to collaborate with researchers                                 | Yes                                                       | 46  | 28.00 |
|                                                                                                                      | No                                                        | 118 | 72.00 |
| The reasons why the TMPs have no willingness (N=118)                                                                 | If secrecy is revealed TM will not be effective           | 102 | 86.00 |
|                                                                                                                      | TM is my means of livelihood (income source)              | 46  | 39.00 |
|                                                                                                                      | No right for royalty or beneficence to reveal the secrecy | 5   | 4.00  |
|                                                                                                                      | I have a promise from my ancestors who taught me TM       | 5   | 4.00  |
|                                                                                                                      | knowledge not to reveal the secrecy                       |     |       |

Key: - TM: Traditional Medicine, MM: Modern Medicine, TMP: Traditional Medicinal practitioners

#### 4. 5. Traditional Medicine Sources, Preparation, container used, Accessibility, and Conservation of TM

Most of the treatment approaches 136 (83%) of the TMPs were plant-based. Crushed and squeezed liquid preparations were the most commonly used herbal preparations 118 (72%), followed by preparations applied to the skin 30 (18.3%). About 77.4% of the TMPs responded that they collect the medicinal plants from the wild forest, and 67% of them reported having difficulties with the accessibility of medicinal plants because of deforestation and agricultural expansion. Only 29% of them conserved the plants in their home gardens, and 6% responded that they control and protect massive harvesting and degradation of wild medicinal plants (Table 4.5).

Table 4.5: Information related to TMP care and services provided, sources, preparation, storage of remedies, accessibility and conservation of MP

| Variables (More than one answer is possible)                                          | Responses                                                            | N   | %     |
|---------------------------------------------------------------------------------------|----------------------------------------------------------------------|-----|-------|
| Of which type do the TMPs serve the community?                                        | As an herbalist (using medicinal plants only),                       | 138 | 84.00 |
|                                                                                       | As a spiritual healer                                                | 22  | 13.00 |
|                                                                                       | As a bone settler                                                    | 39  | 24.00 |
|                                                                                       | As a capping specialist                                              | 5   | 3.00  |
| What are the sources of TMP remedies or approaches that TPMs employ?                  | Plant and plant products                                             | 136 | 83.00 |
|                                                                                       | Animal products                                                      | 48  | 29.00 |
|                                                                                       | Mineral                                                              | 18  | 11.00 |
|                                                                                       | Prayer                                                               | 8   | 5.00  |
|                                                                                       | Holly water( tsebel or Zemzem)                                       | 8   | 5.00  |
| The types of preparation (dosage, formulations) that the TMPs commonly employs for MP | Liquids/solutions                                                    | 118 | 72.00 |
|                                                                                       | Ointments                                                            | 38  | 23.20 |
|                                                                                       | Pellets                                                              | 31  | 19.00 |
|                                                                                       | Powders                                                              | 27  | 16.50 |
|                                                                                       | Steam inhalation                                                     | 39  | 24.00 |
|                                                                                       | Unprocessed plant                                                    | 14  | 9.00  |
|                                                                                       | Other                                                                | 8   | 5.00  |
|                                                                                       | Wrapping in plastics, paper bags, or papers                          | 116 | 70.80 |
|                                                                                       | In containers that have lids with label                              | 23  | 14.04 |
| THs response about storage of remedies that are to be dispensed.                      | As needed                                                            | 10  | 6.10  |
|                                                                                       | Giving fresh plant at hand                                           | 23  | 14.02 |
|                                                                                       | Yes, TM has some negative effect                                     | 108 | 66.00 |
| THs response about TM having drawbacks                                                | No, TM has no weak side                                              | 56  | 34.00 |
|                                                                                       | Problems in the diagnosis of illness                                 | 60  | 56.00 |
|                                                                                       | Use of intangible aspects, sorcery, etc.                             | 46  | 43.00 |
| If so, what are the reasons? (N = 108).                                               | problems of dosage level of Traditional remedies                     | 50  | 46.00 |
|                                                                                       | Harm full practices like child tooth extraction, uvul-ectomy, etc... | 20  | 19.00 |
|                                                                                       | Easily accessed since I collect from garden                          | 38  | 23.00 |
|                                                                                       | Access from the forest without much problem                          | 31  | 19.00 |
|                                                                                       | Difficult to access due to deforestation and agricultural expansion  | 110 | 67.00 |
| THs response to medicinal plant accessibility when compared to the past               | Yes                                                                  | 56  | 34.00 |
|                                                                                       | Not                                                                  | 108 | 66.00 |
|                                                                                       | Cultivation MP as home garden                                        | 16  | 29.00 |
| TMP's response to MP conservation and use                                             | Protecting against deforestation and fire                            | 25  | 45.00 |
|                                                                                       | Controlling of the massive harvest of wild MP                        | 10  | 6.00  |
|                                                                                       | Maintenance of seeding in nurseries, planting, and conservation      | 5   | 9.00  |
| How TMPs conserve and manage MP species(N=56)                                         | Home garden(cultivated plant)                                        | 65  | 65.00 |
|                                                                                       | Collection from local forests(wild collection)                       | 127 | 77.44 |
|                                                                                       | From TM distributors or in the market                                | 7   | 4.30  |

Key; - MP: Medicinal Plant,

### 4.3.6. Responses of TMPs on Future Activities Related to Ethnomedicine Practices

This study also assessed the TMPs' perception and willingness to support ongoing efforts of the Ministry of Health to promote traditional medicine, including training of practitioners, regulation, and integration into the health system. As a result, approximately 82% of practitioners responded positively to the need for TMP training, with the majority (76%) requiring training on dosages. Concerning the ongoing efforts by the Ministry of Health to promote Ethnomedicine, 76% of the practitioners agreed with integration and 73% of them expressed their willingness to support the regulation of ethnomedicine practices (Table 4.6).

Table 4. 6. Responses from TMP about plans for training of TMP, regulation, promotion, and integration of TM with MM

| Variable (More than one answer is possible.)                                                               | Responses                                                    | N   | %     |
|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|-----|-------|
| THs response about the need for training for THs                                                           | Yes                                                          | 134 | 82.00 |
|                                                                                                            | No                                                           | 32  | 18.00 |
| THs response about areas of training needed (N = 134)                                                      | Dosages of TM remedies                                       | 102 | 76.00 |
|                                                                                                            | Hygiene and sanitation during the processing of TM           | 68  | 51.00 |
|                                                                                                            | Conservation, cultivation, and sustainable uses of MP        | 71  | 53.00 |
|                                                                                                            | Other identified                                             | 8   | 6.00  |
| What is TMPs response to the ongoing efforts of the Ministry of Health to regulate and promote TM and why? | Yes, it is a favorable step for TM                           | 122 | 74.00 |
|                                                                                                            | No, it is a step against TM                                  | 23  | 14.00 |
|                                                                                                            | I am not sure, I don't know.                                 | 19  | 12.00 |
| THs responses about their support for the integration of TM with MM                                        | Yes                                                          | 119 | 73.00 |
|                                                                                                            | No                                                           | 45  | 27.00 |
| If so, what is the suggestion of TMPs for integration of TM with MM? (N =119)                              | The TMPs should train MM                                     | 55  | 46.00 |
|                                                                                                            | The health professional must first be trained in TM          | 19  | 16.00 |
|                                                                                                            | The healer must be paid a salary                             | 36  | 30.00 |
|                                                                                                            | Policy and legal framework should be developed and practiced | 38  | 32.00 |

Keys: -TM: Traditional Medicine, MM: Modern Medicine

#### 4.3.7. Comparative Analysis of Current Ethnomedicinal survey and Data from Literature Reviews

Forty-two papers were reviewed to determine the medicinal plants utilized in Ethiopia for the treatment of common human infections. As a result, 150 plant species were identified from 59 plant families. *Calpurnia aurea* (Aiton) Benth and *Croton macrostachyus* Hochst Ex Delile, were the most referenced plant species, with the Fabaceae (18/12%), Asteraceae (13/8.6%), and Cucurbitaceae (9/6%) being the most cited plant families (Table 4.7). The authors compared medicinal plants used for different infections from the current ethnomedicinal study conducted with the literature reviewed across the country. Medicinal plant families, species, plant parts used, routes of administration, and growth habits were compared.

Accordingly, Asteraceae and Fabaceae were the most identified families in both ethnomedicinal studies conducted in the study area, and the literature reviews across the country. In both cases, *C. macrostachyus* Hochst was the most common plant species used for different ailments. Except for the difference in vernacular naming, more than half of the plants were similar in species and their usefulness in the treatment of common human infections (Tables 4.3 and 4.7). From the 70 species studied, 30 plant species (42.9%) were newly reported for their use against currently perceived infections and had no evidence of previous use reported from reviewed literatures.

These plant species were *Crinum abyssinicum* Hochst, *Centella asiatica* (L.), *Vernonia myriantha* Hook.f., *Bidens macroptera* (Sch. Bip. ex Chiov.) Mesfin, *Stereospermum kunthianum* Cham, *Cynoglossum lanceolatum* Forsk., *Coronopus didymus* (L.) Sm, *Canarina eminii* Asch & Schweinf, *Carica papaya* L., *Dracaena steudneri* Engl, *Senna septemtrionalis* (Viv) Irwin & Barneby, *Vigna unguiculata* (L.) Walp., *Erythrina brucei* Schweinf, *Plectranthus edulis* (Vatke) Agnew, *Ocimum basilicum* L., *Clerodendrum myricoides* Hochst, *Linum usitatissimum* L., *Stephania abyssinica* (Quart.- Dill. & A.Rich.) Walp., *Ficus vasta* forssk, *Morus alba* L, *Psidium guajava* L., *Securidaca longipedunculata* Fresen, *Thalictrum rhynchocarpum* Dill, *Nigella sativa* L., *Hagenia abyssinica* (Brace) J.F.Gmel., *Coffea arabica*, *Solanum nigrum* L., *Camellia sinensis*, *Grewia ferruginea* Hochst. Ex A. Rich, and *Aframomum corrorima* (A. Braun) P.C.M. Jansen.

Shrubs and herbs were the dominant plant growth habits. Leaves and roots were the most prevalent plant parts used, and oral, as well as dermal, were the most commonly used routes of administration in both the ethnomedicinal study and literature review results (Fig. 4.3, 4.4 and 4.5). Conversely, information on

indigenous designations of suspected illnesses with their descriptions and perceived causes, knowledge transfer and documentation of ethnomedicinal, and the views of TMPs about future training, regulation, promotion, and integration of ethnomedicinal in to the health system, were obtained in our current study, and the literature search evidence is limited. Intestinal parasites (15/21.3%), gastrointestinal infections (13/19%), skin infections (9/12.9%), respiratory tract infections (8/11.4%), leishmaniasis (6/8.6%), and rabies (6/8.6%) were amongst the most common infections for which these plants were traditionally used.



Table 4.7: Verities of medicinal plants used for common infections by indigenous knowledge in Ethiopia, compiled by literature search

| Family         | Scientific name                                | Local name         | Grow form | Part used | Specific use                                    | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                         |
|----------------|------------------------------------------------|--------------------|-----------|-----------|-------------------------------------------------|-----------------|---------------------|----------------|------------------|-----------------|---------------------------------------------------|
| Asteraceae     | <i>Bidens pilosa</i> L.                        | Not known          | Herb      | L.Se      | Ear infection                                   | Oral, local     | NE                  | NE             | NE               | 1               | Birhanet al 2011                                  |
| Acanthaceae    | <i>Acanthus eminens</i> C. B. Clarke           | Amesa buticho (Sd) | Shrub     | L R       | Wound infection                                 | Oral            | NE                  | NE             | NE               | 1               | Megersa et al.2013                                |
|                | <i>Justicia schimperiana</i> T.Anderson        | Dhumuga (O)        | Shrub     | R/ Bark   | Toothache, Typhoid, STI                         | topical         | NE                  | NE             | Yes              | 3               | (Ayele et al., 2016; Chekole, 2017; Yirga, 2010). |
| Amaranthaceae  | <i>Amaranthus caudatus</i> L.                  | Iyyaasuu           | Herb      | L         | Diarrhea                                        | Oral            | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                              |
|                | <i>Achyranthes aspera</i> L.                   | Derrgu/bagge       | Herb      | R         | Gonorrhea, Blood clotting, Snake bite, Anthrax  | Oral            | NE                  | NE             | NE               | 3               | (Mesfin et al., 2009; Wondimu et al., 2007)       |
| Amaryllidaceae | <i>Allium sativum</i> L.                       | Nechshinkurte (A)  | Herb      | Bulb      | Influenza, Headache, malaria, stomach infection | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                             |
| Anacardaceae   | <i>Rhus retinorrhoea</i> Oliv.                 | Talo enbis         | Tree      | L         | Tonsillitis                                     | Oral            | NE                  | NE             | NE               | 1               | (Chekole, 2017)                                   |
|                | <i>Schinus molle</i> L.                        | Qundoberbere(A)    | Tree      | SB, L     | Toothache                                       | Oral            | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                            |
| Apiaceae       | <i>Foeniculum vulgare</i> Mill.                | Enshilal           | Herb      | A/ p      | Tooth and Stomachache, Kidney Infection,        | Oral            | NE                  | NE             | NE               | 2               | (Chekole, 2017; Wondimu et al., 2007)             |
|                | <i>Acokanthera schimperi</i> (A.DC.) Schweinf. | Qaraaruu           | Herb      | L         | Skin infection, scabies                         | Topical         | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                             |
| Apocynaceae    | <i>Carissa spinarum</i> L.                     | Adanii(O)          | Shrub     | R         | Gonorrhea, Diarrhea                             | Oral            | NE                  | NE             | NE               | 2               | (Mesfin et al., 2014; Tefera and Kim, 2019)       |
|                | <i>Carissa spinarum</i> L.                     | Agamsa             | Shrub     | R         | Gonorrhea, Stomach ache                         | Oral, topical   | NE                  | NE             | NE               | 2               | (Abera Balcha, 2014; Wondimu et al., 2007)        |

Table 4.7. Continued

| Family           | Scientific name                               | Local name               | Grow form   | Part used | Specific use                  | Roots of Adm. | Compound Identified | Clinical study c | Biological test | No. of citation | Reference                                                        |
|------------------|-----------------------------------------------|--------------------------|-------------|-----------|-------------------------------|---------------|---------------------|------------------|-----------------|-----------------|------------------------------------------------------------------|
|                  | <i>Maytenus arbutifolia</i> (A. Rich) Wilczek | Kombollechae             | Shrub       | R         | Wound infection               | Dermal        | NE                  | NE               | NE              | 1               | (Mesfin et al., 2009)                                            |
| Araceae          | <i>Colocasia esculenta</i> (L.) Schott.       | Godarre                  | Herb        | L, R      | Trachoma                      | Oral, ocular  | NE                  | NE               | NE              | 1               | (Mesfin et al., 2009)                                            |
| Aristolochiaceae | <i>Aristolochia bracteolata</i> Lam.          | Abujenajil               |             | Se        | Diarrhea                      | Oral          | NE                  | NE               | NE              | 1               | (Flatie et al., 2009)                                            |
| Asclepiadaceae   | <i>Gomphocarpus fruticosus</i> (L.) Ait. f.   | Haazmalaa                | Herb        | L         | Anthrax                       | Oral          | NE                  | NE               | NE              | 1               | (Wondimu et al., 2007)                                           |
|                  | <i>Gomphocarpus purpurascens</i> A. Rich      | Mexxino                  | Shrub       | R, SB     | Febrile illness               | Oral          | NE                  | NE               | NE              | 1               | (Mesfin et al., 2009)                                            |
|                  | <i>Kanahala laniflora</i> (Forssk.) R. Br.    | Wundiffo                 | Shrub       | R, L      | Bronchitis, Hepatitis         | Oral          | NE                  | NE               | NE              | 1               | (Mesfin et al., 2009)                                            |
| Asparagaceae     | <i>Asparagus setaceus</i> Lam.                | Saritii                  | Climber     | L, R      | UTI                           | Oral, Topical | NE                  | NE               | NE              | 1               | (Wondimu et al., 2007)                                           |
|                  | <i>Asparagus africanus</i> L.                 | . Uffae                  | Shrub       | R         | Wound, UTI                    | Oral          | NE                  | NE               | NE              | 1               | (Mesfin et al., 2009)                                            |
| Asteraceae       | <i>Vernonia schimperi</i> DC.                 | Hecho (Sd), Gerawa (Amh) | Herb        | L, R, SE  | Wound, GI infection           | Oral, Topical | NE                  | NE               | NE              | 1               | (Tefera and Kim, 2019)                                           |
|                  | <i>Vernonia amygdalina</i> Delile             | Ebicha                   | Shrub       | L, R      | Diarrhea, GI infection        | Oral, Topical | NE                  | NE               | Yes             | 3               | (Mesfin et al., 2009; Wondimu et al., 2007)(Mesfin et al., 2014) |
|                  | <i>Echinops longisetus</i> A. Rich.           | Mata bokke(O)            | Shrub(wild) | L         | different forms of infections | Topical, oral | NE                  | NE               | NE              | 1               | (Ayele et al., 2016)                                             |

Table 4.7. Continued

| Family       | Scientific name                               | Local name   | Grow form | Part used | Specific use                               | Roots of admin.  | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                         |
|--------------|-----------------------------------------------|--------------|-----------|-----------|--------------------------------------------|------------------|---------------------|----------------|------------------|-----------------|---------------------------------------------------|
| Asteraceae   | <i>Carduus schimper</i> Sch. Bip. ex A. Rich. | Kosheshila   | Herb      | R         | Fibril illness                             | Oral             | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                             |
|              | <i>Conyza schimper</i> Sch. Bip. ex A. Rich   | Inshlal      | Herb      | Se        | Febrile illness                            | Nasal            | NE                  | NE             | NE               | 1               | (Chekole, 2017)                                   |
|              | <i>Echinops kebericho</i> , Mesfin            | Qabarichoo   | Herb      | L         | Toothache                                  | Oral             | NE                  | NE             | NE               | 2               | (Abera Balcha, 2014; Mesfin et al., 2014)         |
|              | <i>Cirsium englerianum</i> O. Hoffm.          | Galigloo     | Herb      | R         | Skin infection                             | Dermal           | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                             |
|              | <i>Xanthium strumarium</i> L.                 | Dehanekayae  | Herb      | R, L      | Wart, Skin, infection                      | Dermal           | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                             |
|              | <i>Helianthus annuus</i> L.                   | Suffae       | Herb      | All part  | Food poison                                | Oral             | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                             |
|              | <i>Xanthium strumarium</i> L.                 | Dehanekayae  | Herb      | All part  | Skin infection                             | Dermal           | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                             |
|              | <i>Acmella caulirhiza</i> Del.                | Gutichaa     | Herb      | F         | Tonsillitis                                | Oral             | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                              |
| Balanitaceae | <i>Balanites aegyptiaca</i> (L.) Delile       | Godicho (Sd) | Tree      | Fe        | diarrhea, GI infection                     | Oral             | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                            |
| Boraginaceae | <i>Cordia africana</i> Lam.                   | Waddeessa(O) | Tree      | R, SB, F  | Different infection, Toothache             | Oral             | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                             |
|              | <i>Cynoglossum amplifolium</i> Hochst. ex DC. | Ziyad        | Herb      | L         | Eye infection<br>Cataract, wound infection | Topical(ocular ) | NE                  | NE             | NE               | 2               | (Mirutse Giday et al., 2009; Tuasha et al., 2018) |

Table 4.7. Continued

| Family        | Scientific name                            | Local name          | Grow form | Part used | Specific use                                | Roots of admin. | Compound Identified | Clinical study | Biological test | No. of citation | Reference                                                         |
|---------------|--------------------------------------------|---------------------|-----------|-----------|---------------------------------------------|-----------------|---------------------|----------------|-----------------|-----------------|-------------------------------------------------------------------|
| Brassicaceae  | <i>Ehretia cymosa</i> Thonn                | Ulaagaa             | Tree      | R         | GI infection                                | Oral            | NE                  | NE             | NE              | 1               | (Wondimu et al., 2007)                                            |
|               | <i>Brassica nigra</i> (L.) Koch            | Sinafich            | Herb      | Se        | Abdominal pain                              | Oral            | NE                  | NE             | NE              | 1               | (Amsalu et al., 2018)                                             |
|               | <i>Lepidium sativum</i> L                  | Feto/feexxoo        | Herb      | Se        | Diarrhea, GI infection RTI                  | Oral            | NE                  | NE             | NE              | 2               | (Amsalu et al., 2018; Mesfin et al., 2009)                        |
| Capparidaceae | <i>Boscia salicifolia</i> Olive.           | Tisha               | T         | L         | Ear infection                               | Oral            | NE                  | NE             | NE              | 1               | (Chekole, 2017)                                                   |
|               | <i>Crateva adansonii</i> D.C. Prodr        | Qolladii            | Shrub     | R         | Gonorrhea                                   | Oral            | NE                  | NE             | NE              | 1               | (Abera Balcha, 2014)                                              |
|               | <i>Capparis fascicularis</i> DC            | Haarangamaaa adii   | Shrub     | R         | Toothache                                   | Oral, Topical   | NE                  | NE             | NE              | 1               | (Wondimu et al., 2007)                                            |
|               | <i>Capparis tomentosa</i> Lam.             | Haarangamaag uraach | Shrub     | R         | Wounds infection<br>Toothache, GI infection | Oral, Topical   | NE                  | NE             | NE              | 2               | (Tefera and Kim, 2019; Wondimu et al., 2007)                      |
|               | <i>Maerua angolensis</i> DC.               | Qanqalchaa          | Tree      | R         | STI                                         | Oral            | NE                  | NE             | NE              | 1               | (Wondimu et al., 2007)                                            |
| Celastraceae  | <i>Ritchiea albersii</i> Gilg              | Arbuu               | Tree      | SB        | Coughwound infection                        | Oral            | NE                  | NE             | NE              | 1               | (Abera Balcha, 2014)                                              |
|               | <i>Maytenus senegalensis</i> (Lam.) Excell | Shekko              | Shrub     | R         | Febrile Disease                             | Oral            | NE                  | NE             | NE              | 1               | (Mesfin et al., 2009)                                             |
| Combretaceae  | <i>Combretum molle</i> R.Br. ex G.Don      | Akuma(T)            | T         | Bark      | Anti-Leishmaniasis, diarrhea, stomach pain  | Oral            | NE                  | NE             | Yes             | 3               | (Asres, et al., n.d.; Nibret and Wink, 2011; Zenebe et al., 2012) |
|               | <i>Terminalia brownii</i> Fresen.          | Olpata              | Tree      | R/L/S B   | Anthrax wound infection                     | Topical, oral   | NE                  | NE             | NE              | 1               | (Mesfin et al., 2009)                                             |

Table 4. 7 Continued

| Family         | Scientific name                             | Local name           | Grow form | Part used | Specific use                                          | Roots of admin.     | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                  |
|----------------|---------------------------------------------|----------------------|-----------|-----------|-------------------------------------------------------|---------------------|---------------------|----------------|------------------|-----------------|--------------------------------------------|
| Commelinaceae  | <i>Commelina benghalensis</i> L.            | Laaluunxe            | Herb      | L         | Skin infection                                        | Topical             | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)               |
|                | <i>Commelina africana</i> L.                | Lalonxe (Sd)         | Herb      | SB, L     | Skin infection                                        | Topical             | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                     |
| Convolvulaceae | <i>Ipomoea batatas</i> (L.) Lam.            | siquar-dinich/dammee | Herb      | L         | Boil                                                  | Topical             | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)               |
| Crassulaceae   | <i>Kalanchoe petitiata</i> A. Rich.         | Andahula/Endohahila  | Herb      | L/SB, R   | Trachoma, syphilis, and different swellings<br>Tonsil | Dermal, Nasal       | NE                  | NE             | NE               | 2               | (Amsalu et al., 2018; Tadege et al., 2005) |
|                | <i>Kalanchoe densiflora</i> Rolfe           | Endahula             | Herb      | L         | Gonorrhea                                             | Dermal              | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                       |
|                | <i>Momordica foetida</i> Schumacher         | Sambao               | climber   | L         | Toothache<br>Wound                                    | Oral/<br>dermal     | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                      |
| Curcubitaceae  | <i>Zehneria scabra</i> (Linn.f.) Sond.      | Hareg resa/harresa   | Herb      | L /f      | Febrile illness, Wound infection<br>Liver problem     | Dermal, nasal, oral | NE                  | NE             | NE               | 1               | (Chekole, 2017)                            |
|                | <i>Cucurbita pepo</i> L.                    | Buqqee               | Herb      | Se        | Gonorrhea, Skin infection                             | Oral                | NE                  | NE             | NE               | 2               | (Abera Balcha, 2014; Mesfin et al., 2009)  |
|                | <i>Lagenaria siceraria</i> (Molina) Standl. | Botto                | Herb      | Fi        | Gonorrhea                                             | Oral                | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                      |

Table 4. 7 Continued

| Family         | Scientific name                                 | Local name               | Grow form | Part used | Specific use                                                         | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. citation | Reference                                                                                                                   |
|----------------|-------------------------------------------------|--------------------------|-----------|-----------|----------------------------------------------------------------------|-----------------|---------------------|----------------|------------------|--------------|-----------------------------------------------------------------------------------------------------------------------------|
|                | <i>Cucumis prophetarum</i> L.                   | Arado/Holato             | Climber   | R         | Gonorrhea                                                            | Oral            | NE                  | NE             | NE               | 1            | (Wondimu et al., 2007)                                                                                                      |
|                | <i>Cucumis ficifolius</i> A. Rich.              | Yafer geranger,          | Herb      | R         | GI infection, Jaundice, anthrax                                      | Oral            | NE                  | NE             | NE               | 1            | (Teka et al., 2015)                                                                                                         |
|                | <i>Peponium vogelii</i> (Hook.f.) Engl          | Basu baaqula             | Climber   | Fe        | STI                                                                  | Oral            | NE                  | NE             | NE               | 1            | (Tuasha et al., 2018)                                                                                                       |
| Ebenaceae      | <i>Euclea racemosa</i> subsp. schimper (A. DC.) | Me'esaa                  | Shrub     | R         | Kidney infection                                                     | Oral, Topical   | NE                  | NE             | NE               | 1            | (Wondimu et al., 2007)                                                                                                      |
|                | <i>Euphorbia abbyssinica</i> J.F. Gmel.         | Adaamii                  | Tree      | SB        | GI infection, STD, Leishmaniasis                                     | Oral, dermal    | NE                  | NE             | NE               | 1            | (Abera Balcha, 2014)                                                                                                        |
|                | <i>Clutia abyssinica</i> Jaub. and Spach        | Fiyele-fej, Maalaasincho | Shrub     | Stem      | Dysentery, Meningitis                                                | Oral            | NE                  | NE             | NE               | 2            | (Amsalu et al., 2018; Tuasha et al., 2018)                                                                                  |
| Euphorbiaceae, | <i>Croton macrostachyus</i> Hochst. Ex Delile   | Bakaniisa(O)             | Tree      | L SB      | GI infection, wound infection, Leishmaniasis, Gonorrhea, TB, anthrax | Oral Dermal     | NE                  | NE             | NE               | 6            | (Abera Balcha, 2014; Chekole, 2017; Mesfin et al., 2014; Nibret and Wink, 2011; Tefera and Kim, 2019; Wondimu et al., 2007) |
|                | <i>Euphorbia candelabrum</i> Kotschy            | Haadaamii                | Tree      | SB        | Gonorrhea                                                            | Oral            | NE                  | NE             | NE               | 1            | (Wondimu et al., 2007)                                                                                                      |
|                | <i>Euphorbia tirucalli</i> L                    | Qincibii                 | Shrub     | SB        | Gonorrhea                                                            | Oral            | NE                  | NE             | NE               | 1            | (Wondimu et al., 2007)                                                                                                      |
|                | <i>Ricinus communis</i> L.                      | Qoboo                    | shrub     | Fe        | Gonorrhea                                                            | Oral            | NE                  | NE             | NE               | 1            | (Wondimu et al., 2007)                                                                                                      |

Table 4.7 Continued

| Family   | Scientific name                                   | Local name            | Grow form | Part used  | Specific use                                                                                                          | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                                                                                                              |
|----------|---------------------------------------------------|-----------------------|-----------|------------|-----------------------------------------------------------------------------------------------------------------------|-----------------|---------------------|----------------|------------------|-----------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Fabaceae | <i>Tragia brevipes</i> Pax                        | Sonicho (Sd)          | Herb      | L, Fe      | Anthrax, Diarrhea                                                                                                     | Oral            | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                                                                                                                 |
|          | <i>Albizia malacophylla</i> (A. Rich)             | Jebiya(H)             | Tree      | SB         | RTI                                                                                                                   | Oral            | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                                                                                                           |
|          | <i>Calpurnia aurea</i> (Ait.) Benth               | Digita(H) Ceekaa (O)  | Tree      | Root, SB   | Diarrhea, Abdominal infection, Tooth, infection, TB, dysentery, syphilis, tapeworm, trachoma, scabies, elephantiasis, | Oral, Topical   | NE                  | NE             | NE               | 6               | (Abera Balcha, 2014; Mesfin et al., 2009; Mirutse Giday et al., 2009; Tefera and Kim, 2019; Wondimu et al., 2007; Zenebe et al., 2012) |
|          | <i>Calpurnia subdecandra</i> (L'Herit.) Schweick. | Not known             | Shrub     | Leaf       | Unspecified skin disease                                                                                              | Dermal          | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                                                                                                                   |
|          | <i>A. gumifera</i> (J.F.Gmel.) C. A. Sm           | Ambabeesssa(O)        |           | RB & SB L, | Leishmaniasis anti-helminthic (tapeworm), antifungal, anti trypanosomal, anti Leishmaniasis                           | Oral dermal     | NE                  | NE             | NE               | 3               | (Chekole et al., 2015; Tefera and Kim, 2019) (Giday et al., 2007)                                                                      |
|          | <i>Acacia abyssinica</i> Hochst. ex Benth.        | Sondii(O)             | Tree      | L          | Leishmaniasis, skin infection, GI infection, Scorpion poison                                                          | Dermal          | NE                  | NE             | NE               | 2               | (Chekole et al., 2015; Tefera and Kim, 2019)                                                                                           |
|          | <i>Indigofera spicata</i> Forssk.                 | Shersherit,           | Herb      | R          | AFI                                                                                                                   | Oral            | NE                  | NE             | NE               | 1               | (Giday et al., 2007)                                                                                                                   |
|          | <i>Millettia ferruginea</i> (hochst.) Baker.      | Sotalloo (O)/ Birbira | Tree      | R          | Gangrene, Skin infection                                                                                              | Topical         | NE                  | NE             | NE               | 2               | (Amsalu et al., 2018; Ayele et al., 2016)                                                                                              |
|          | <i>Senna occidentalis</i> (L.) Link               | Assenmeka             | Herb      | R          | Gonorrhea, Tonsillitis                                                                                                | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                                                                                                                  |
|          | <i>Indigofera spicata</i> Forssk.                 | kursi-asht            | Herb      | All part   | Boil, Meningitis                                                                                                      | Oral, Topical   | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                                                                                                           |

Table 4. 7. Continued

| Family       | Scientific name                                         | Local name    | Grow form | Part used | Specific use                             | Roots of admin.            | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                    |
|--------------|---------------------------------------------------------|---------------|-----------|-----------|------------------------------------------|----------------------------|---------------------|----------------|------------------|-----------------|------------------------------|
| Fabaceae     | <i>Trifolium rueppellianum</i> Fresen                   | tikus-asht    | Herb      | All part  | Meningitis                               | Oral<br>Topical            | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009) |
|              | <i>Acacia albida</i> Del.                               | Garbii(o)     | Tree      | All part  | Diarrhea                                 | Oral                       | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
|              | <i>Acacia etbaica</i> Schweinf                          | Doddottii(o)  | Tree      | L         | Tonsillitis, gonorrhea                   | Oral                       | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
|              | <i>Acacia nilotica</i> (L.) Willd ex. Del.              | Qordimo       | Tree      | SB        | Tonsillitis                              | Topical                    | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
|              | <i>Acacia oerfota</i> (Forssk.) Schweinf                | Ajo           | Shrub     | SB        | Toothache                                | Oral                       | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
|              | <i>Acacia tortilis</i> (Forssk.) Hayne                  | Xadetchaa     | Tree      | L         | Tonsillitis                              | Oral                       | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
|              | <i>Senna didymobotrya</i> (Fresen.) H.S.Irwin & Barneby | Chebicha (Sd) | Shrub     | L         | Wound infection, eye infection, diarrhea | Oral<br>Topical,<br>Ocular | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)       |
|              | <i>Trigonella foenum-graecum</i> L                      | Sunqo(O)      | Herb      | Se, R, SB | Abdominal infection, Amoeba              | Oral                       | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)       |
|              | <i>Calpurnia subdecandra</i> (L'Herit.) Schweick        |               |           | L         | Skin infection                           | Dermal                     | NE                  | NE             | NE               | 1               | ((Abera Balcha, 2014)        |
| Gentianaceae | <i>Enicostema axillare</i> (Lam.) A. Rich.              | Doddoto       | Herb      | R         | Anthrax                                  | Oral                       | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)       |
| Icacinaceae  | <i>Apodytes dimidiata</i> E. Mey. ex Arn.               | Gefe          | SB        | R L       | Cholera                                  | Oral                       | NE                  | NE             | Yes              | 1               | (Tefera and Kim, 2019)       |



Table 4.7 Continued

| Family       | Scientific name                                           | Local name      | Grow form     | Part used | Specific use                              | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                    |
|--------------|-----------------------------------------------------------|-----------------|---------------|-----------|-------------------------------------------|-----------------|---------------------|----------------|------------------|-----------------|----------------------------------------------|
| Lamiaceae    | <i>Leonotis ocymifolia</i> (Burm F.) A.                   | Feres zeng      | Herb          | Fr., L    | Diarrhea                                  | Oral            | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                        |
|              | <i>Meriandra dianthera</i> (Roth ex Roem & schult.) Briq. | Mentese         | Shrub         | F         | Trachoma                                  | Ocular          | NE                  | NE             | NE               | 1               | (Chekole, 2017)                              |
|              | <i>Ocimum gratissimum</i> L.                              | Damakase(O)     | Herb          | L         | Dysentery, AFI                            | Oral            | NE                  | NE             | NE               | 2               | (Amsalu et al., 2018; Tefera and Kim, 2019)  |
|              | <i>Leucas deflexa</i> Hook. f.                            | Qechemen        | Herb          | L         | Diarrhea                                  | Oral            | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                 |
|              | <i>Leucas abyssinica</i> L                                | Asaalee         | Herb          | R, SB     | Tonsillitis, Eye disease                  | Oral            | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                       |
|              | <i>Premna schimper</i> Engl                               | Udo (Sd)        | Shrub         | L, R, SB  | Abdominal infection, Tooth infection, RTI | Oral, Topical   | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                       |
|              | <i>Pycnostachys abyssinica</i> Fresen                     | Yeeroo          | Shrub         |           | Abdominal pain, moch                      | Topical, oral   | Yes                 | NE             | Yes              | 1               | (Tegene Tesfaye Tole and Lomi Abayneh, 2020) |
|              | <i>Satureja punctata</i> (Benth.) Briq.                   | Amesa (Sd)      | Herb          | L         | Skin infection                            | Topical         | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                       |
|              | <i>Salvia nilotica</i> Juss. ex Jacq.                     | Kot jebesa (Sd) | Herb          | L, R, SB  | AFI, skin disease, toothache              | Oral, Topical   | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                       |
| Loranthaceae | <i>Buddleja polystachya</i> Fresen.                       | Hanfaaree       | Herb          | L         | Eye disease                               | Ocular          | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                         |
|              | <i>Oncocalyx kellri</i> (Engl.) M. Gilbert                | Haarmobandaa    | hemiparasitic | L         | Wound infection                           | Topical         | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                       |
|              | <i>Oncocalyx schimperi</i> (A. Rich.) M. Gillbert         | Haarmo-goraa    | hemiparasitic | L         | Wound infection                           | Topical         | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                       |
| Lythraceae   | <i>Punica granatum</i> L                                  | Roman           | Tree          | L         | Diarrhea                                  | Topical         | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                       |

Table 4. 7. Continued

| Family         | Scientific name                             | Local name   | Grow form | Part used | Specific use                                                                           | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                                    |
|----------------|---------------------------------------------|--------------|-----------|-----------|----------------------------------------------------------------------------------------|-----------------|---------------------|----------------|------------------|-----------------|--------------------------------------------------------------|
| Malvaceae      | <i>Malva parviflora</i> L.                  | Lit          | Herb      | R         | Asthma, wound infection                                                                | Oral, Topical   | NE                  | NE             | NE               | 1               | (Tadeg et al., 2005)                                         |
|                | <i>Grewia villosa</i> Willd.                | Bururi(O)    | Shrub     | R, L      | Gonorrhea, Diarrhea                                                                    | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                        |
|                | <i>Sida schimperiana</i> Hochst. ex A.Rich. | Not known    | Shrub     | R, L, F   | Dysentery, Influenza, Liver disease                                                    | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                        |
|                | <i>Gossypium arboreum</i> L.                | Jirbiae      | Shrub     | R         | Lymphatic swelling, skin infection                                                     | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                                        |
|                | <i>Sida rhombifolia</i> L.                  | Not stated   | Herb      | R         | Abdominal pain, amoebiasis                                                             | Oral            | NE                  | NE             | Yes              | 1               | (Teka et al., 2015)                                          |
| Meliaceae      | <i>Melia azedarach</i> L.                   | Kininii      | Tree      | SB        | Diarrhea                                                                               | Oral            | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                                       |
|                | <i>Ekebergia capensis</i> parm.             | Oloncho (Sd) | Tree      | L, SB     | TB, anthrax                                                                            | Oral, Topical   | NE                  | NE             | NE               | 1               | (Tefera and Kim, 2019)                                       |
| Melanthaceae   | <i>Bersama abyssinica</i> Fresen.           | Lolchiisaa   | Tree      | L, SB, Se | Wound infection, Bronchitis, Febrile illness, Dandruff, wound, burn infection, scabies | Dermal, Oral    | NE                  | NE             | Yes              | 3               | (Abera Balcha, 2014; Mesfin et al., 2009; Teka et al., 2015) |
| Menispermaceae | <i>Cissampelos pareira</i> L.               | Abujelajil   | Shrub     | R         | Diarrhea, GI infection                                                                 | Oral            | NE                  | NE             | NE               | 1               | (Flatie et al., 2009)                                        |
| Myrsinaceae    | <i>Embelia schimperi</i>                    | Habquu       | Tree      | FE        | Different infection                                                                    | oral            | NE                  | NE             | Yes              |                 | (Lulekal et al., 2014)                                       |
|                | <i>Maesa lanceolata</i> Forssk.             | Abbayii(O)   | Tree      | SB        | Skin infection, Elephantiasis                                                          | Dermal          | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                                         |
|                | <i>Syzygium guineense</i> (Willd.) DC.      | Duwancho     | Tree      | R, L      | Gonorrhea,                                                                             | Oral            | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                        |

Table 4.7. Continued

| Family          | Scientific name                                               | Local name     | Grow form | Part used | Specific use                                          | Roots of admin. | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                         |
|-----------------|---------------------------------------------------------------|----------------|-----------|-----------|-------------------------------------------------------|-----------------|---------------------|----------------|------------------|-----------------|---------------------------------------------------|
| Oleaceae        | <i>Olea europaea</i> L. subsp. Cuspidate Wall. Ex G. Don) Cif | Ejersa(O)      | Tree      | St        | Toothache, Trachoma                                   | Oral            | NE                  | NE             | NE               | 2               | ((Amsalu et al., 2018; Tuasha et al., 2018)       |
| Phytolacca ceae | <i>Phytolacca dodecandra</i> , L ‘Hert.                       | Andoodee       | Shrub     | L, R      | Sinusitis, Febrile illness                            | Oral, Nasal     | NE                  | NE             | NE               | 2               | (Mesfin et al., 2009)                             |
| Pittosporaceae  | <i>Pittosporum viridiflorum</i> Sis                           | Hunbosho       | Herb      | L R       | TB, pneumonia                                         | Oral            | NE                  | NE             | YEs              | 1               | (Teka et al., 2015)                               |
| Polygonaceae    | <i>Rumex abyssinicus</i> Jacq.                                | Dhangago (O)   | Herb      | R         | Gonorrhea                                             | Oral            | NE                  | NE             | Yes              | 1               | (Ayele et al., 2016)                              |
| Poaceae,        | <i>Cynodon nlemfuensis</i> Vanderyst                          | Coqorsa        | Herb      | L, SB     | Tonsillitis                                           | Dermal,         | NE                  | NE             | NE               | 1               | ((Abera Balcha, 2014)                             |
| Polygonaceae    | <i>Rumex nepalensis</i> Spreng.                               | Tullet (A)     | Herb      | L         | Fungal infection, dysentery, Toothache, Eye infection | Topical, Oral   | NE                  | NE             | NE               | 2               | (Mesfin et al., 2014; Mirutse Giday et al., 2009) |
|                 | <i>Rumex nervosus</i> Vahl                                    | Dhangagoo      | Shrub     | L, R      | Diarrhea, Wound infection Hepatitis                   | Oral, Topical   | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                            |
| Ranunculaceae   | <i>Ranunculus multifidus</i> Forssk                           | chadera        | Herb      | L         | Eye infection                                         | Topical         | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                      |
|                 | <i>Clematis hirsuta</i> Guill. & Perr.                        | Azo-hareg      | Climber   | Leaf      | Leishmaniasis Wound infection, Trachoma               | Topical/ Ocular | NE                  | NE             | NE               | 2               | (Mirutse Giday et al., 2009; Yirga, 2010)         |
|                 | <i>Clematis longicauda</i> steud ex.A. rich                   | Edda fitie (O) | Climber   | L         | Tonsillitis                                           | Oral            | NE                  | NE             | Yes              | 1               | (Ayele et al., 2016)                              |

Table 4. 7. Continued

| Family      | Scientific name                              | Local name | Grow form | Part used | Specific use                                    | Roots admin.          | of | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                                             |
|-------------|----------------------------------------------|------------|-----------|-----------|-------------------------------------------------|-----------------------|----|---------------------|----------------|------------------|-----------------|-----------------------------------------------------------------------|
| Rhamnaceae  | <i>Rhamnus prinoides</i> L'Herit             | Gesho      | Shrub     | Se, L     | Tonsillitis<br>Skin infection<br>Dysentery, STI | Oral<br>Topical Nasal |    | NE                  | NE             | NE               | 2               | (Amsalu et al., 2018; Tuasha et al., 2018)                            |
| Rosaceae    | <i>Prunus africana</i> (Hook. f.) Kalkman    | Hoomii     | Tree      | R, SB     | Ear infection, toothache, GI infection          | Oral, Local           |    | NE                  | NE             | NE               | 3               | (Abera Balcha, 2014; Mesfin et al., 2009; Mirutse Giday et al., 2009) |
|             | <i>Rubus apetalus</i> Poir.                  | Gora       | Shrub     | R         | TB                                              | Oral                  |    | NE                  | NE             | NE               | 1               | (Tuasha et al., 2018)                                                 |
| Rubiaceae   | <i>Gardenia ternifolia</i> Schumach. & Thonn | Bodut      | Tree      | SB        | AFI                                             | Oral, Nasal           |    | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                                          |
|             | <i>Pentas lanceolata</i> (Forssk.) Deflers   | Tigoch     | Herb      | L         | Lymphadenitis, Boil, Meningitis                 | Oral                  |    | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                                          |
| Rutaceae    | <i>Clausena anisata</i> (Wild.) Benth.       | Ulumaa'i   | Tree      | L         | Skin infection                                  | Topical, Nasal        |    | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                                                  |
|             | <i>Ruta chalepensis</i> L.                   | Xenadamae  | Herb      | L         | GI infection, Toothache                         | Oral                  |    | NE                  | NE             | NE               | 2               | (Mesfin et al., 2009; Wondimu et al., 2007)                           |
| Santalaceae | <i>Osyris quadripartita</i> Decn             | Qerets(H)  | Shrub     | L         | Unspecified infection                           | Topical, Nasal        |    | NE                  | NE             | NE               | 1               | (Giday et al., 2007)                                                  |
| Sapindaceae | <i>Dodonaea angustifolia</i> L.f.            | Itach (O)  | Shrub     | L         | Skin infection                                  | Oral                  |    | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                                 |

Table 4.7. Continued

| Family           | Scientific name                            | Local name             | Grow form | Part used | Specific use                                                                                 | Roots admin.         | of | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                                                                                |
|------------------|--------------------------------------------|------------------------|-----------|-----------|----------------------------------------------------------------------------------------------|----------------------|----|---------------------|----------------|------------------|-----------------|----------------------------------------------------------------------------------------------------------|
| Scrophulariaceae | <i>Verbascum sinaiticum</i> Benth          | Guraa-haaree, Ketetina | Shrub     | L         | Anti-Leishmaniasis, Diarrhea, anthrax, elephantiasis, measles, superficial fungal infections | Oral, Topical, Nasal |    | NE                  | NE             | NE               | 3               | (Mesfin et al., 2009; Tadege et al., 2005; Wondimu et al., 2007)                                         |
| Simaroubaceae    | <i>Brucea antidysenterica</i> Swiss Chard. | Avalo(T)               | Shrub     | R, L, Se  | Wound infection, Leishmaniasis, Bloody diarrhea                                              | Oral, Dermal         |    | NE                  | NE             | NE               | 4               | (Amsalu et al., 2018; Elizabeth Elizabeth et al., 2014; Mesfin et al., 2014; Mirutse Giday et al., 2009) |
| Smilacaceae      | <i>Smilax anceps</i> Willd.                | yarep                  | Climber   | R         | Ear infection                                                                                | Local                |    | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)                                                                             |
|                  | <i>Nicotiana tabacum</i> L.                | Machara(O)             | Herb      | L         | Diarrhea, Gonorrhea,                                                                         | Oral                 |    | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                                                                    |
| Solanaceae       | <i>Solanum incanum</i> L.                  | Embuay                 | Shrub     | R, L      | Gonorrhea. Rabies,                                                                           | Oral                 |    | NE                  | NE             | NE               | 1               | (Mesfin et al., 2014)                                                                                    |
|                  | <i>Capsicum annuum</i> L.                  | Qaria                  | Herb      | Fr.       | Dysentery, vomiting                                                                          | Oral                 |    | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                                                                                    |
|                  | <i>Withania somnifera</i> (L.) Dunal       | Gizewa                 | shrub     | L         | Fibril illness                                                                               | Dermal, Nasal        |    | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                                                                                    |
|                  | <i>Solanum glabratum</i> Dunala            | Darguuwaraabesaa       | Herb      | R         | Anthrax                                                                                      | Oral                 |    | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                                                                                   |

Table4. 7. Continued

| Family         | Scientific name                                              | Local name      | Grow form | Part used  | Specific use                                                       | Roots admin.  | of | Compound Identified | Clinical study | Bioactivity test | No. of citation | Reference                                  |
|----------------|--------------------------------------------------------------|-----------------|-----------|------------|--------------------------------------------------------------------|---------------|----|---------------------|----------------|------------------|-----------------|--------------------------------------------|
|                | <i>Datura stramonium</i> L.                                  | Asangra(O)      | Herb      | L,Se       | teeth infection, stomach pain, skin infection                      | Topical oral  |    | NE                  | NE             | NE               | 2               | (Abera Balcha, 2014; Tefera and Kim, 2019) |
|                | <i>Solanum anguivai</i> Lam.                                 | Ambu            | Herb      | L          | Lymphadenitis                                                      | Topical       |    | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)               |
| Urticaceae     | <i>Pouzolzia parasitica</i> (Forssk.) Schweinf.              | charun-asht     | Herb      | L          | Lymphadenitis                                                      | Oral, Topical |    | NE                  | NE             | NE               | 1               | (Mirutse Giday et al., 2009)               |
|                | <i>Lippia adoensis</i> Hochst. ex Walp                       | Kossoret/kusaye | Herb      | L          | superficial bacterial & fungal infections                          | Topical       |    | NE                  | NE             | NE               | 1               | (Tadeg et al., 2005)                       |
| Verbenaceae    | <i>Verbena officinalis</i> L.                                | Atuch           | Herb      | R          | Abdominal pain, diarrhea, stomachache                              | Oral          |    | NE                  | NE             | NE               | 1               | (Amsalu et al., 2018)                      |
|                | <i>Lantana camara</i> L.                                     | Yewef kollo     | Shrub     | SB         | Diarrhea                                                           | Oral          |    | NE                  | NE             | NE               | 1               | (Mesfin et al., 2009)                      |
|                | <i>Lippia adoensis</i> Hochst. ex Walp. var. <i>adoensis</i> | Kessie          | Herb      | L          | Abdominal pain, abdominal bloating, jaundice, anthrax, indigestion | Oral, Topical |    | NE                  | NE             | Yes              | 1               | (Teka et al., 2015)                        |
| Vitaceae       | <i>Cissus quadrangularis</i> L.                              | Gaalee-aabdii   | Climber   | R          | Toothache Gonorrhea                                                | Oral          |    | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                     |
|                | <i>Cissus rotundifolia</i> (Forssk.) Vahl Gale               | Gale            | Climber   | L          | Gonorrhea                                                          | Oral          |    | NE                  | NE             | NE               | 1               | (Wondimu et al., 2007)                     |
| Zingiberaceae, | <i>Zingiber officinale</i> Roscoe                            | Zinjibila       | Herb      | L, S, bulb | Influenza GI infection                                             | Oral          |    | NE                  | NE             | NE               | 1               | (Abera Balcha, 2014)                       |

Keys: - AFI= Acute febrile illness, BU=Bulb, Fe fruit, Fi-Flower, GI GastroIntestinal, H= Amharic language. Tig =Tigre Language, HP = hemiparasitic L= leaf, NE= No Evidence, O=Oromiffa language, R= Root, S= shrub, SB-Steem Bark, Sd.= Sidama language, Se-Seed, STI= sexual transmitted disease, T=tree, UTI= Urinary Tract Infection, WP all part,

#### 4.4. Discussion

Through an ethnomedicinal study and literature search, the authors compiled detailed information on medicinal plants used for the treatment of common human illnesses and related indigenous knowledge. The study identified various plant families and species that have been utilized to treat human illnesses.

Regarding the socioeconomic characteristics of TMPs, the majority of TMPs were males aged  $60 \pm 13$  years, who live in a rural area, and earned a very low income. This is in line with similar studies in other parts of the country (9,11). According to TMP response the younger generations appear uninterested in ethnomedicinal practice. It is therefore; clear that unless medicinal plant information is collected and documented, the knowledge and practice will disappear together over time. The lower income of TMPs also shows the economic benefit of herbal medicine is insignificant in Ethiopia.

Traditional Medicinal Practitioners and communities in the study area have developed different knowledge and perspectives on how to designate different infections based on different cultural and spiritual beliefs over time. Some indigenous designations, perceptions of the causes, and treatment modalities are related to modern medicinal theories of infections. For example, as presented in Table 4.2, the communities, as well as TMPs, explained poor personal hygiene can cause scabies, onchocerciasis, and intestinal parasites. However, some perceptions about the causes of diseases, such as TB and liver cirrhosis, were completely different from their scientific etiologies, and need to be corrected through training. Except for the language and some designation differences, this study is in line with traditional beliefs and disease healing-practices of other Ethiopian communities (11).

Asteraceae, Fabaceae, Euphorbiaceae, and Lamiaceae were the most identified plant families, while *V. amygdalina*, *C. macrostachyus*, *O. gratissimum* L., and *P. dodecandra* L. (Hert.) were the most commonly used plant species for the treatment of human infections. These medicinal plants are mostly used for the treatment of intestinal parasites, skin, respiratory tract, sexually transmitted, and gastrointestinal infections, rabies, tonsillitis, and neglected tropical diseases, such as leishmaniasis. These findings are in line with some similar studies in the country (12,13); but the plant species identified, were different from those identified in a study conducted in the eastern parts of the country (14). The differences may be due to geographic differences and in the perception of illness of the TMPs.

Pig and hedgehog meat were also the most commonly mentioned wild animal species for the treatment of different infections, including TB and HIV/AIDS. Leaves and roots were the most commonly used plant parts, while oral, as well as dermal, were the most commonly used routes of administration. This is in line with a similar study conducted in Punjab province, Pakistan (15). Shrubs and herbs were the most commonly used plant growth forms, in accordance with the study conducted by Megersa and his colleagues (2013), as well as that of Balcha (2014). From studies conducted in Cameroon, Tamil Nadu, and Kashmir, the dominant plant parts used in the preparation of remedies were leaves (16–18), and this was also the case in this study.

The majority of the oral preparations of medicinal plants were mixed with water, coffee, honey, and milk, whereas the dermal preparations were mostly mixed with butter and honey. Coffee and honey were used as medication and as mixing solvents. This is in line with the study by Suleman and colleagues (2018) (19). However, in nations that were comparatively developed with advanced technologies, as well as ethnomedicinal research and development, such as India, the majority of remedies are administered in the form of juice, paste, poultice, IV infusion, decoction, and exudates (16).

The communities preferred ethnomedicine to modern medicine because of affordability and readily availability; and perceived to be free of adverse effects (20,21). Most (79%) of the TMPs reported that they inherited their knowledge from family members and needed to orally transfer their knowledge only to their children. This is also similar to the findings of previous studies (6,10,14). The younger generation's willingness to become TMPs was very minimal because they perceived it to be an outdated practice, which is no basis in modern science.

Documentation of the service provided by traditional healers was almost negligible. Majority of TMPs (77.4%) reported that they collected medicinal plants from the wild, while others (67%) reported difficulties in obtaining these plants because of deforestation and agricultural expansion. The review by Kala et al. (2006) (22) also identified similar difficulties in Northern India. Continuous exploitation of several medicinal plant species from the wild and substantial loss of their habitats have resulted in the decline of the population of many high-value medicinal plant species in India (22). Similarly, agricultural expansion, overgrazing, firewood collection, mining, and cutting trees for construction and furniture were the major threats to medicinal plants in the Gozamin district, East Gojjam, Amhara Region (23).



A few TMPs (29%) conserve the plants in their home gardens, and very few (6%) control and protect the massive harvesting and degradation of wild medicinal plants. This study also assessed the willingness of TMPs to support the ongoing effort of the Ministry of Health to promote ethnomedicinal research and development. Some TMPs responded confidently that they are willing to reveal all the secrets of their ethnomedicinal knowledge and to collaborate with researchers to evaluate the safety, efficacy, and quality of medicinal plants. According to this study, the main reasons for most respondents' unwillingness to divulge ethnomedicinal information are the following: 1) fear of the remedies losing effectiveness if the secret is revealed, and 2) being their source of income; both of which align with similar studies (24,25). Most of the TMPs have an encouraging outlook on their need for training; most need training on dosages remedies, hygiene, and sanitation during the processing of the medicine. This finding is in line with the result of similar study conducted (12-14).

Thirty plant species were newly reported and identified for their use against perceived infections in the study area without any evidence from a literature search. This is more than those reported by Rashid and colleagues (26). One hundred and fifty medicinal plant species were identified from 42 reviewed articles.

The majority of the studies were inventory types; of which only 5% of the plant species were tested. This is quite different from the study conducted by Mahomoodally et al (2018)(5). The growth habits of medicinal plants reviewed were herbal plants (42%) and shrubs (28%). More than half (59.4%) of the plant parts reviewed were leaves, followed by roots (35%). Oral administration was the major route of administration (76%), followed by dermal (36.7%). Furthermore, certain promising starts were made in the investigation of bioactivity and compound isolation from medicinal plants in Ethiopia. *A. abyssinica* Sch. Bip, leaf aqueous extract was found to be the most effective, with the lowest MIC against *Listeria monocytogenes* (0.2 µl/ml), *S. aureus* (0.2 µl/ml), *P.*

*aeruginosa* (1.6 µl/ml), and *K. pneumonia*. Therefore, the above mentioned plant species will be promising candidates for further investigation of bioactive secondary metabolites( 12, 13)

#### **4.5. Conclusion and Recommendations**

Totally 164 TMPs identified by this study. Most of the TMPs were males, all are older than 60 years and live in rural areas. The TMPs and the communities use their own local language and culture to diagnose the perceived causes of infections. Certain indigenous designations and perceptions of the causes of some infections are consistent with modern medicine theories of infections. The Asteraceae, and Fabaceae, were among the most commonly used plant families, while *V. amygdalina* Delile, *C. macrostachyus* Hochst, and *O. gratissimum* L were the most commonly reported plant species. Leaves and roots were the most frequently used plant parts, with oral and dermal routes of administration being the most frequently used.

Traditional Medicinal Practitioners used a wide variety of ethnomedicinal plants for the treatment of perceived human infections. Furthermore, nationwide gathering and documenting of associated indigenous knowledge and practices in Ethiopia should be a priority, given that the majority of TMPs are of an older generation. Destruction of indigenous medicinal plants should be banned, and the plants conserved through the establishment of botanical gardens. The findings from this study have laid the foundation for further researchs. Furthermore, scientific validation of the efficacy, safety, and quality of medicinal plants is very important and should be a priority area.

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**CHAPTER V:**  
**PHYTOCHEMICAL SCREENING, ANTIMICROBIAL**  
**ACTIVITY AND ANTIBIOTIC MODULATING EFFECT**  
**EVALUATION OF SELECTED MEDICINAL PLANTS IN**  
**ETHIOPIA**

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## **Abstract**

**Background:** The emergence and spread of resistant microbes continue to be a major public health concern. Effective treatment alternatives, particularly from traditionally used medicinal plants, are needed.

**Objectives:** The main objective of this study was to conduct phytochemical screening and antimicrobial activity evaluation of selected traditionally used medicinal plants in Ethiopia.

**Methods:** Phytochemical classes of compounds were screened using different standard methods. Minimum inhibitory concentrations were measured using the broth micro-dilution method against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 21.0 and the findings were presented descriptively and using non parametric one-way ANOVA analysis (Kruskal-Wallis/Dunn's test).

**Results:** The phytochemical constituents identified were flavonoids, alkaloids, glycosides, phenols, saponins, steroids, and terpenoids, with flavonoids, alkaloids, and phenols being most abundantly visible. The crude extracts and chloroform fractions of the extracts showed activity against the tested strains. The crude extract of *Thalictrum rhynchocarpum* Quart. -Dill. & A.Rich root demonstrated superior activity against all the tested strains with the lowest minimum inhibitory concentrations of 0.48 µg/ml against *Staphylococcus aureus* and *Escherichia coli*; 0.98 µg/ml against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*; and 3.90 µg/ml against *Candida albicans*.

**Conclusion:** The majority of the evaluated medicinal plants demonstrated remarkable activity against tested microbial strains, which can be attributed to the presence of secondary metabolites of different classes of compounds. The findings provided scientific evidence for the use of these traditionally used medicinal plants.

**Keywords:** Traditional medicine; medicinal plants; phytochemical screening; antimicrobial activity; Minimum Inhibitory Concentration.



## 5.1. Introduction

The burden of morbidity and mortality related to microbial infections has been inclined towards developing countries due to the increased emergence and spread of drug-resistant microbes (1-4). Antibiotics that were once thought to be miracle cures are now unable to treat resistant bacteria (5, 6,7). Over recent years, the number of new approved antimicrobial medicines has dropped greatly, and the supply of effective antimicrobials is anticipated to run out shortly (5–7). Traditionally used medicinal plants represent the ancient and remain an indispensable source of novel and effective pharmaceutical products. The capacity to use active substances derived from plants or their synthetic equivalents in medicine has improved with the development of phytochemistry and pharmaceutical chemistry (Reference?).

Africa has an immensely rich biodiversity and knowledge base in the use of plants to treat various ailments, including infectious diseases. However, these resources have hardly been investigated scientifically. In Ethiopia, some of the studies presented on medicinal plants were limited to an ethnomedicinal survey, and the results were listed with incomplete descriptions (8,9). In various regions of Ethiopia different plant species are traditionally utilized for the treatment and prevention of both human and animal illnesses (10–14). Therefore, it is of paramount importance to focus on antimicrobial drug discovery from medicinal plants, particularly from those which are widely used by TMPs for the mitigation of infectious diseases.

## 5.2. Materials and Methods

Qualitative phytochemical screening and *in-vitro* antimicrobial investigation were conducted from 1<sup>st</sup> June to 31<sup>st</sup> September 2021. Criteria for plant selection, methods of extraction, phytochemical screening, antimicrobial activity tests, plant extracts, and conventional antibiotic combination tests were listed under Chapter III, Sections 3.4–3.7.

### 5.3. Results

#### 5.3.1. The Ethnomedicinal Information of Selected Plants

Among the twelve plant species selected, five (42%) were trees and three (25%) were herbs. The roots of the plants were the most commonly used, followed by stem bark. The selected plant species were usually utilized to treat different perceived infections. *Justicia schimperiana*, *A. gumifera*, *S. nigrum*, *C. abyssinicum* traditionally used for the treatment of neglected tropical diseases such as leishmaniasis, trypanosomiasis, onchocerciasis and scabies. *C. hirsuta*, *C. macrostachyus*, and *T. rhynchocarpum* were claimed to be used for the treatment of gastrointestinal infections. And *C. abyssinicum*, *D. steudneri* and *M. foetida* were used for the treatments of wound infections. The majorities of the TMPs provide the plant materials as fresh, crushed or powdered and applied on the affected part or administered orally by mixing them with milk, honey, butter, coffee, or water (Table 5.1).

Table 5.1: Ethnomedicinal information of the selected medicinal plants.

| S. N | Family name   | Plant species                                                | Local name          | Growth | Part used       | Disease treated                                                                                                                    | Preparation                                                                                                                                          | Voucher No.  |
|------|---------------|--------------------------------------------------------------|---------------------|--------|-----------------|------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| 1    | Acanthaceae   | <i>J. schimperiana</i><br>(Hochst. ex Nees)<br>(Acanthaceae) | Dhumuga<br>a        | H      | R, SB,<br>L & F | Leishmaniasis, skin infection, Onchocerciasis,<br>sudden mental illness (likift)                                                   | Crushed leaves or roots are mixed with butter and<br>hot water and applied to the affected skin and tied<br>around the head.                         | P01/20<br>21 |
| 2    | Euphorbiaceae | <i>C. macrostachys</i><br>Hochst. ex A. Rich                 | Bakkaniissa         | T      | R SB &<br>L,    | GI infection, AFI (malaria-like illness), fungal and<br>bacterial skin infections, STD, insect replant,<br>allergy, herpes zoster, | Crushed dried powder is mixed with water and<br>honey and administered orally. The leaf is rubbed<br>on the affected skin, and the smoke is inhaled. | P02/20<br>21 |
| 3    | Fabaceae      | <i>A. gumifera</i><br>(J.F.Gmel.) C. A. Sm,                  | ambabeesssa         | T      | RB &<br>SB L,   | Anti-helminthic (tapeworm), antifungal, anti<br>trypanosomal, anti-Leishmaniasis                                                   | Crushed dried powder is taken orally.                                                                                                                | P03/20<br>21 |
| 4    | Ranunculaceae | <i>C. hirsuta</i> Guill. &<br>Perr.                          | Idda naachaa        | Cl     | WP              | antifungal anti trypanosomal, anti-Leishmaniasis<br>skin infection                                                                 | applied to the affected skin                                                                                                                         | P04/20<br>21 |
| 5    | Sapindaceae   | <i>D. angustifolia</i> L.f.                                  | Kitkita/<br>Ittacha | T      | L               |                                                                                                                                    | The crushed and dried powder is taken orally and<br>applied to the skin.                                                                             | P05/20<br>21 |
| 6    | Solanaceae    | <i>S.nigrum</i> L.                                           | Iddii adii          | H      | F               | Anti-rabies, anti-Leishmaniasis, anti-bacteria                                                                                     | Apply crushed fresh fruit or dried fruit powder to<br>the affected skin.                                                                             | P06/20<br>21 |

Table 5.1: continued

| S. N | Family name    | Plant species                                    | Local name             | Growth | Part used | Disease treated                                                                                    | Preparation                                                                     | Voucher No   |
|------|----------------|--------------------------------------------------|------------------------|--------|-----------|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------|
| 7    | Amaryllidaceae | <i>C. abyssinicum</i><br>Hochst. ex A. Rich      | Qullubii<br>warabessaa | H      | R, L      | Leishmaniasis wound infection, cancrroid,<br>hypertension, diabetes, cancer...                     | Root crushed and applied                                                        | P07/20<br>21 |
| 8    | Dracaenaceae   | <i>D. steudneri</i> Engl.                        | Sarxee                 | T      | R, L      | Lymphadenitis, wound infection, Leishmaniasis,<br>likift, elephantiasis                            | The leaf burned and applied on the affected site                                | P08/20<br>21 |
| 9    | Lamiaceae      | <i>P. abyssinica</i> Fresen                      | yeeroo                 | H      | R, L      | Sudden mental illness (likift), different infections,<br>scabies, fungal infections, elephantiasis | Lease massaged with the affected area                                           | P09/20<br>21 |
| 10   | Meliaceae      | <i>T. dregae</i> Sand.                           | Luuyaa                 | T      | Sb, L     | Bacterial infection, Leishmaniasis,                                                                | Crushed powder applied on the affected site or<br>swallowed with water or honey | P10/20<br>21 |
| 11   | Cucurbitaceae  | <i>M. foetida</i> Schumach.<br>et Thonn          | Saramba'oo             | Cl     | R, L      | wound infection, clotting, snake bite,                                                             | Fresh leaf crushed and applied                                                  | P11.20<br>21 |
| 12   | Ranunculaceae  | <i>T. rhynchocarpum</i><br>Quart.-Dill. & A.Rich | Serebezu               | H      | R         | different infections, malaria                                                                      | Fresh root chewed, juice boiled and taken orally                                | P12/20<br>21 |

Key:- Cl: Climber, F: fruit, H: Herb, L: Leaf, R: Root, SB: Stem bark, T: Tree, WP Whole Part, GI, AFI, STD, IP intestinal Parasites

### 5.3.2. Phytochemical Screening

All the selected plant extracts were presented with notable positive phytochemical results (Table 5.2), which were evidenced with remarkable color changes. Flavonoids, alkaloids and phenols were the most abundant classes of compounds in the majorities of the screened plants. Flavonoids were exhibited highly positive with significantly visible color change in *J. schimperiana* root,

*C. macrostachyus* stem bark, *A. gumifera* stem bark, *C. hirsuta* whole part, *T. dregae* stem bark, *C. abyssinicum* root bulb and *T. rhynchocarpum* root. Alkaloid was the next most class of compound which presented in *C. macrostachyus*, *C. hirsute*, *C. abyssinicum* and *T. rhynchocarpum* root. Phenols were the third phytochemicals presented in *J. schimperiana*, *C. macrostachyus*, *A. gumifera*, *C. hirsute*, *C. abyssinicum* and *T. rhynchocarpum* root. Thin layer chromatography also confirmed the presence of different phytochemical components.

Table 5.2: Phytochemical screening results of crude extract of selected plant species

| .N | Plant species extracts                               | Secondary metabolites test results |           |            |         |          |          |            |         | TLC spots (Visible) |
|----|------------------------------------------------------|------------------------------------|-----------|------------|---------|----------|----------|------------|---------|---------------------|
|    |                                                      | Flavonoids                         | Alkaloids | Glycosides | Phenols | Saponins | Steroids | Terpenoids | Tannins |                     |
| 1  | <i>J. schimperiana</i> (Hochst. ex Nees) (Root /     | +++                                | +++       | ++         | +++     | +        | ++       | ++         | ++      | Many                |
| 2  | <i>C. macrostachys</i> Hochst (Stem bark)            | +++                                | +++       | ++         | +++     | ++       | ++       | ++         | ++      | Many                |
| 3  | <i>A. gumifera</i> (J.F.Gmel.) C. A. Sm(Stem bark)   | +++                                | +++       | ++         | +++     | ++       | ++       | ++         | ++      | Many                |
| 4  | <i>C. hirsuta</i> Guill. & Perr(Whole part)          | +++                                | +++       | ++         | ++      | +++      | ++       | +++        | -       | >3                  |
| 5  | <i>S.nigrum</i> L(Fruit)                             | ++                                 | ++        | +++        | +       | ++       | ++       | ++         | +++     | 2                   |
| 6  | <i>D. angustifolia</i> L.f.( Leaf)                   | ++                                 | ++        | +          | ++      | +        | +        | +          | ++      | 5                   |
| 7  | <i>C. abyssinicum</i> Hochst. ex A. Rich (Root bulb) | +++                                | +++       | +++        | +++     | +++      | ++       | ++         | -       | 5                   |
| 8  | <i>T. dregae</i> Sand. (Stem bark)                   | ++                                 | ++        | ++         | +++     | ++       | ++       | ++         | ++      | 3                   |
| 9  | <i>D. steudneri</i> Engl. (Root)                     | ++                                 | +++       | ++         | -       | +        | ++       | ++         | +++     | 5                   |
| 10 | <i>M. foetida</i> Schumach. et Thonn (Leaf)          | ++                                 | +++       | ++         | +       | ++       | ++       | ++         | ++      | 4                   |
| 11 | <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich (Root)  | +++                                | ++        | +++        | +++     | +        | ++       | ++         | ++      | 5                   |
| 12 | <i>P. abyssinica</i> Fresen                          | +                                  | +         | -          | +       | -        | -        | +          | ++      | invisible           |

Key: (+) mildly positive, (++) moderately Positive, and (+++) highly positive (significantly visible color change)

### 5.3.3. Antimicrobial Activities

Among the selected plant species, all fractions of the extracts from *T. rhynchocarpum* root presented with the greatest efficacy against all tested strains (Table 5.3). Among the selected plant species, all fractions of the extracts from *T. rhynchocarpum* root presented with the greatest efficacy against all tested strains. Particularly the crude extract of *T. rhynchocarpum* exhibited with MIC 0.98 µg/ml against *K. pneumoniae* and *P. aeruginosa* and 0.48 µg/ml against *S. aureus* and *E. coli*. Furthermore, extracts from *J. schimperiana* and *C. macrostachyus* also demonstrated remarkable activity against tested microbial strains. The chloroform fraction of *J. schimperiana* root presented the highest activity with MIC of 3.8 µg/ml against *S. aureus* and *E. coli*. The crude extract of *C. macrostachys* exhibited 3.9 µg/ml against *K. pneumoniae*, 7.8 µg/ml against *S. aureus*, and *E. coli*. The chloroform fraction of *C. macrostachys* also demonstrated the lowest MIC with 7.8 µg/ml against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. A Kruskal-Wallis/Dunn's statistical test showed a significant difference between the tested samples and fractions of the plant extracts on MIC with tested plant species ( $H(df:11) = X^2:180.45, p=0.000$ )).

Table 5.3: Antimicrobial activities test of selected plant crude extract and different solvent fraction

| Plant species extract                          | Fraction of the plant extract | Antimicrobial activities (MIC in µg/ml) |       |       |       |       |
|------------------------------------------------|-------------------------------|-----------------------------------------|-------|-------|-------|-------|
|                                                |                               | KP                                      | PA    | SA    | EC    | CA    |
| <i>Justicia schimperiana</i> (Hochst. ex Nees) | Crude extract                 | 15.6                                    | 7.8   | 7.8   | 7.8   | 15.6  |
|                                                | n-hexane fraction             | 31.25                                   | 15.6  | 7.8   | 7.8   | 31.25 |
|                                                | Chloroform fraction           | 15.6                                    | 7.8   | 3.8   | 3.8   | 15.6  |
|                                                | Methanol fraction             | 15.6                                    | 7.8   | 15.6  | 15.6  | 31.25 |
| <i>C. macrostachys</i> Hochest                 | Crude extract                 | 31.25                                   | 31.25 | 7.8   | 7.8   | 31.25 |
|                                                | n-hexane fraction             | 62.5                                    | 62.5  | 15.6  | 15.6  | 31.25 |
|                                                | Chloroform fraction           | 31.25                                   | 31.25 | 15.6  | 15.6  | 62.5  |
|                                                | Methanol fraction             | 15.6                                    | 15.6  | 7.8   | 7.8   | 31.25 |
| <i>A. gumifera</i> (J.F.Gmel.) C. A. Sm        | Crude extract                 | 3.9                                     | 3.9   | 7.8   | 7.8   | 7.8   |
|                                                | n-hexane fraction             | 31.25                                   | 125   | 31.25 | 31.25 | 31.25 |
|                                                | Chloroform fraction           | 15.60                                   | 15.6  | 15.6  | 15.6  | 31.25 |
|                                                | Methanol fraction             | 31.25                                   | 31.25 | 62.50 | 62.5  | 62.5  |
| <i>C. hirsuta</i> Guill. & Perr                | Crude extract                 | 7.8                                     | 7.8   | 15.6  | 15.6  | 15.6  |
|                                                | n-hexane fraction             | 7.8                                     | 7.8   | 15.6  | 15.6  | 31.25 |
|                                                | Chloroform fraction           | 7.8                                     | 7.8   | 7.8   | 7.8   | 15.6  |
|                                                | Methanol fraction             | 31.25                                   | 31.25 | 15.6  | 31.25 | 62.5  |



Table 5.3: continued

| Plant species extracts                   | Fraction of the plant extract | Antimicrobial activities (MIC in µg/ml) |       |        |        |        |
|------------------------------------------|-------------------------------|-----------------------------------------|-------|--------|--------|--------|
|                                          |                               | KP                                      | PA    | SA     | EC     | CA     |
| <i>S. nigrum</i> L.                      | Crude extract                 | 15.6                                    | 31.25 | 250    | 250    | 62.5   |
|                                          | n-hexane fraction             | 62.5                                    | 250   | 250    | 250    | 125    |
|                                          | Chloroform fraction           | 62.5                                    | 62.5  | 62.5   | 125    | 125    |
|                                          | Methanol fraction             | 125                                     | 125   | 125    | 250    | 62.5   |
| <i>D. angustifolia</i> L.f.              | Crude extract                 | 15.6                                    | 15.6  | 31.25  | 31.25  | 31.25  |
|                                          | n-hexane fraction             | 62.5                                    | 62.5  | 62.5   | 31.25  | 31.25  |
|                                          | Chloroform fraction           | 15.6                                    | 15.6  | 62.5   | 31.25  | 31.25  |
|                                          | Methanol fraction             | 15.6                                    | 7.8   | 62.5   | 62.5   | 62.5   |
| <i>C. abyssinicum</i> Hochst. ex A. Rich | Crude extract                 | 15.6                                    | 15.6  | 7.8    | 7.8    | 15.6   |
|                                          | n-hexane fraction             | 31.25                                   | 31.25 | 7.8    | 7.8    | 15.6   |
|                                          | Chloroform fraction           | 7.8                                     | 7.8   | 7.8    | 15.6   | 15.6   |
|                                          | Methanol fraction             | 31.25                                   | 31.25 | 31.25  | 31.25  | 31.25  |
| <i>D. steudneri</i> Engl.                | Crude extract                 | 225                                     | 125   | 125    | 125    | 125    |
|                                          | n-hexane fraction             | 500                                     | >500  | >500   | >500   | >500   |
|                                          | Chloroform fraction           | 225                                     | 225   | 125    | 125    | 62.5   |
|                                          | Methanol fraction             | 225                                     | 225   | 225    | 225    | 225    |
| <i>P. abyssinica</i> Fresen              | Crude extract                 | 62.5                                    | 62.5  | 31.25  | 31.25  | 31.25  |
|                                          | n-hexane fraction             | 125                                     | 125   | 125.00 | 125.00 | 125.00 |
|                                          | Chloroform fraction           | 62.5                                    | 62.5  | 31.25  | 31.25  | 62.5   |
|                                          | Methanol fraction             | 31.25                                   | 31.25 | 15.6   | 15.6   | 62.5   |

Table 5.3: continued

| Plant species                                  | Fraction of the plant extract | Antimicrobial activities (MICin µg/ml) |      |      |      |      |
|------------------------------------------------|-------------------------------|----------------------------------------|------|------|------|------|
|                                                |                               | KP                                     | PA   | SA   | EC   | CA   |
| <i>T. dregae</i> Sand.                         | Crude extract                 | 250                                    | 250  | 125  | 125  | 62.5 |
|                                                | n-hexane fraction             | 125                                    | 125  | 62.5 | 62.5 | 62.5 |
|                                                | Chloroform fraction           | 125                                    | 125  | 250  | 250  | 250  |
|                                                | Methanol fraction             | 125                                    | 125  | 250  | 250  | 250  |
| <i>M. foetida</i> Schumach. et Thonn           | Crude extract                 | 250                                    | 250  | 125  | 250  | 500  |
|                                                | n-hexane fraction             | 500                                    | 500  | 500  | 125  | 500  |
|                                                | Chloroform fraction           | 15.6                                   | 15.6 | 62.5 | 125  | 125  |
|                                                | Methanol fraction             | 125.00                                 | 125  | 125  | 125  | 125  |
| <i>T. rhyncho</i> carpum Quart.-Dill. & A.Rich | Crude extract                 | 0.98                                   | 0.98 | 0.48 | 0.48 | 3.9  |
|                                                | n-hexane fraction             | 7.8                                    | 7.8  | 3.9  | 3.9  | 7.8  |
|                                                | Chloroform fraction           | 1.95                                   | 1.95 | 0.98 | 0.98 | 3.9  |
|                                                | Methanol fraction             | 3.9                                    | 3.9  | 1.95 | 1.95 | 3.9  |
| Gentamycin                                     |                               | 6.25                                   | 12.5 | 1.5  | 3.13 |      |
| clotrimazole                                   |                               |                                        |      |      |      | 10   |

Key KP: *K.pneumoniae*, PA: *P. aeruginosa*, SA: *S.aureus*, EC: *E. coli*, CA: *C.albican*, Avrade

#### **5.3.4. Antibiotic Modulating effects of Selected Plants against *P. aeruginosa* (Atcc 27853) and *S. aureus* (Atcc43300)**

The MIC exhibited by each antibiotic and plants extracts were combined to determine the combination effects against *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC43300). The MIC of amoxicillin alone was 500 µg/ml against *P. aeruginosa* (ATCC 27853) and 250 µg/ml against *S. aureus* (ATCC43300). However, amoxicillin activity was increased 8 times (MIC=62.5 µg/ml) when combined with *C. abyssinicum* Hochst. ex A. Rich extract against *P. aeruginosa* (ATCC 27853). Amoxicillin and *T. rhynchocarpum* Quart.-Dill. & A.Rich combination showed MIC of 7.8 µg/ml against both *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC43300) with its efficacy increased by 64 and 32 times against each strain respectively (Table 5.4)

Table 5.4: Synergistic antibiotic modulating effects of selected three medicinal plants against against

*P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC43300)

| Plant material                                | Fraction   | Tested microbials                 |                              |
|-----------------------------------------------|------------|-----------------------------------|------------------------------|
|                                               |            | <i>P. aeruginosa</i> (ATCC 27853) | <i>S. aureus</i> (ATCC43300) |
|                                               |            | 7.8 µg/ml                         | 7.8 µg/ml                    |
| <i>C. abyssinicum</i> Hochst. ex A. Rich      | chloroform | 7.8 µg/ml                         | 7.8 µg/ml                    |
| <i>C. hirsuta</i> Guill. and Perr             | chloroform | 0.98 µg/ml                        | 0.98 µg/ml                   |
| <i>T. rhynchocarpum</i> Quart.-Dill. & A.Rich | Crude      |                                   |                              |
| Amoxicillin                                   |            | >500 µg/ml                        | 250 µg/ml                    |
| Cloxacillin                                   |            | 250 µg/ml                         | 125 µg/ml                    |
| Tetracycline                                  |            | 62.5 µg/ml                        | 62.5 µg/ml                   |

| Table 5.4: continued                                       |                                   |                              |                                 |
|------------------------------------------------------------|-----------------------------------|------------------------------|---------------------------------|
|                                                            | <i>P. aeruginosa</i> (ATCC 27853) | <i>S. aureus</i> (ATCC43300) | Increase efficacy of PA/SA)by-- |
| Amoxicillin + <i>C. abyssinicum</i> Hochst. ex A. Rich     | 62.5 µg/ml                        | 62.5 µg/ml                   | 8*/4*                           |
| Amoxicillin + <i>C. hirsuta</i> Guill. and Perr            | 15.6 µg/ml                        | 31.25 µg/ml                  | 32*/8*                          |
| Amoxicillin + <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich | 7.8 µg/ml                         | 7.8 µg/ml                    | 64*/32*                         |
| Cloxacillin+ <i>C. abyssinicum</i> Hochst. ex A. Rich      | 31.25 µg/ml                       | 31.25 µg/ml                  | 8*/4                            |
| Cloxacillin+ <i>C. hirsuta</i> Guill. and Perr             | 15.6 µg/ml                        | 31.25 µg/ml                  | 16*/8*                          |
| Cloxacillin+ <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich  | 15.6 µg/ml                        | 15.6 µg/ml                   | 16*/8*                          |
| Tetracycline + <i>C. abyssinicum</i> Hochst. Ex A. Rich    | 31.25 µg/ml                       | 31.25 µg/ml                  | 2*                              |
| Tetracycline+ <i>C. hirsuta</i> Guill. And Perr            | 15.6 µg/ml                        | 15.6 µg/ml                   | 4*8*                            |
| Cloxacillin+ <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich  | 15.6 µg/ml                        | 15.6 µg/ml                   | 16*/8*                          |

Table 5.4: continued

| FIC                                                                                   | <i>P. aeruginosa</i><br>(ATCC27853) | <i>S. aureus</i><br>(ATCC43300) | Interpretations      |
|---------------------------------------------------------------------------------------|-------------------------------------|---------------------------------|----------------------|
| MIC of Amoxicillin + <i>C. abyssinicum</i> Hochst. Ex A. Rich/ MIC of Amoxicillin     | 0.125                               | 0.25                            | Synergistic          |
| MIC of Amoxicillin + <i>C. irsute</i> Guill. And Perr/MIC os Amoxicillin              | 0.031                               | 0.125                           | Synergistic          |
| MIC of Amoxicillin + <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich/ Mic of Amoxicillin | 0.016                               | 0.031                           | Synergistic          |
| MIC of Cloxacillin+ <i>C. abyssinicum</i> Hochst. Ex A. Rich/ MIC of Cloxacillin      | 0.129                               | 0.25                            | Synergistic          |
| MIC of Cloxacillin+ <i>C. 110hirsutea</i> Guill. And Perr?MIC ofCloxacillin           | 0.062                               | 0,25                            | Synergistic          |
| MIC of Cloxacillin+ <i>T.rhynchocarpum</i> Quart.-Dill. & A.Rich/MIC of Cloxacillin   | 0.031                               | 0.062                           | Synergistic          |
| MIC of Tetracycline + <i>C. abyssinicum</i> Hochst. ex A. Rich/MIC of Tetracycline    | 0.5                                 | 0.5                             | Additive             |
| MIC of Tetracycline+ <i>C. hirsuta</i> Guill. And Perr/MIC of Tetracycline            | 0.25                                | 0.5                             | Synergistic/additive |
| MIC of Tetracycline+ T.rhynchocarpum Quart.-Dill. & A.Rich/MIC of Tetracycline        | 0.125                               | 0.125                           | Synergistic          |

Key: AB: antibiotics FIC: fractional inhibitory concentration, PA: *P. aeruginosa*, SA: *S. aureus*

#### 5.4. Discussion

This study identified potential plant species traditionally utilized to treat a variety of infections, including neglected tropical infections, gastrointestinal, skin, and wound infections. The majority of the investigated plants were found to contain different phytochemicals including flavonoids, alkaloids, phenols, glycosides, and steroids; which was confirmed by TLC results presented with multiple spots at different RF values. Among the ~~some~~ classes of compounds flavonoids, alkaloids and phenols were the phytochemicals with significant visible color changes. *Justicia schimperiana*, *C. macrostachyus*, *A. gumifera*, *C. hirsuta*, *T. dregae*, *C. abyssinicum* and *T. rhynchocarpum* were the plant species containing flavonoids, alkaloids and phenols. This finding is similar to the findings of other studies elsewhere (12,15–17).

As illustrated in Table 5.3, most of the evaluated plant extracts demonstrated remarkable activity against selected microbial strains, with the lowest *in-vitro* inhibitory concentration ( $<10\ \mu\text{g/ml}$ ). The crude extracts of *T. rhynchocarpum* root demonstrated the greatest activity, with the lowest MIC of  $0.48\ \mu\text{g/ml}$  against *S. aureus* and *E. coli* and  $0.98\ \mu\text{g/ml}$  *K. pneumonia* and *P. aeruginosa*. The finding is consistent with reports indicating the antimicrobial efficacy of this medicinal plant for treatment of microbial infections (16,18). The chloroform fraction of *J. schimperiana* also demonstrated antibacterial activity with the lowest MIC value of  $3.8\ \mu\text{g/ml}$  against *S. aureus* and *E. coli*. Except *K. pneumoniae* and *C. albican* its MIC is less than  $10\ \mu\text{g/ml}$ , which in line with other similar studies (16,19–21). Extracts from *C. macrostachyus* also exhibited activity against *S. aureus* and *E. coli*. These findings are consistent with previous report that the plants have antimicrobial activity (14,22).

Extracts from *A. gumifera*, *D. angustifolia*, *C. abyssinicum*, *P. abyssinica*, and *C. hirsuta* showed moderate activity against tested microbial strains, with MIC values ranging from  $10\ \mu\text{g/ml}$  to  $100\ \mu\text{g/ml}$ , which is comparable to previous studies (23,24). In contrast with some previous studies, extracts from *S. nigrum*, *D. steudneri*, *T. dregae*, and *M. foetida* showed insignificant activity against tested strains (25–27). The difference could probably be due to differences in preparation methods, the season of plant collection, and/or environmental variations.

The tested plant extracts showed difference in activity between each fraction. Most studied plants' crude extracts and chloroform fractions were found to be more effective against the tested strains of microbes. A crude extract of *T. rhynchocarpum* presented with the greater activity with MIC of 0.48 µg/ml against *S. aureus* and *E. coli* and 0.98 µg/ml against *K. pneumoniae* and *P. aeruginosa*. *Justicia schimperiana* crude extract was more active against

*P. aeruginosa*, *S. aureus*, and *E. coli* with MIC of 7.8 µg/ml. *Thalictrum rhynchocarpum* chloroform fractions exhibited the lowest MIC: 0.98 µg/ml against *S. aureus*, and *E. coli*; 1.95 µg /ml against *K. pneumoniae* and *P. aeruginosa* and 3.9 µg/ml against *C. albican*. Similarly, other studies have shown the presence of differences in activities of the different solvent fractions. Some of the phytochemical components such as terpenoids, alkaloids, flavonoids, and phenols were more extracted in the chloroform fraction, which exhibited the highest activity and broadest spectrum of antimicrobial activities against *S. aureus*, *P. aeruginosa*, and *E. coli* (25,28–30).

All of the crude plant extracts included in this study contained one or more secondary metabolites. Therefore, the observed biological activity profile could be due to either the individual class of compounds present in each plant or the synergistic effect of each class of compounds (29,31,32).

A Kruskal-Wallis H statistical test showed that there was significant difference between the tested plant species with  $H$  (df: 11) = ( $X^2$  :180.45,  $p$  = 0.000) and fractions of the plant extracts  $H$  (df: 3),  $X^2$  = 7.44,  $p$  = 0.059 on MIC. But the difference in microbial strains has no significant association with the difference in MIC of the extracts.

As illustrated in Table 5.4, the MICs of each antibiotic and plant extract were combined to determine the combination effects against *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 43300). The plant extract and antibiotic combination exhibited a promising fractional inhibitory concentration (FIC) index. All the three antimicrobials (tetracycline, amoxicillin, and cloxacillin) with three plant extracts (*C. abyssinicum* Hochst. ex A. Rich, *C. hirsuta* Guill. and Perr, and *T. hynchocarpum* Quart. -Dill. & A. Rich crude extract) showed the FIC value from

0.031 to 0.5 except tetracycline and *C. abyssinicum* Hochst. ex A. Rich (FIC = 0.5), all combination of tested plant extract antibiotics demonstrated synergistic with effect  $FIC < 0.5$ .



Tetracycline and *C. abyssinicum* Hochst. ex A. Rich (FIC = 0.5) exhibited additive effect. The amoxicillin activity was enhanced by 8, 32, and 64 folds against *P. aeruginosa* (ATCC 27853) when combined with *C. abyssinicum* Hochst. ex A. Rich, *C. hirsuta* Guill, Perr, and *T. rhynchoarpum* Quart.-Dill. & A. Rich times, respectively. Combination of cloxacillin with *C. abyssinicum* Hochst. ex A. Rich, *C. hirsuta* Guill and Perr and *T. Rhynchoarpum* Quart.- Dill. & A. Rich increase its efficacy by 8, 16, and 16 folds against *P. aeruginosa* (ATCC 27853) and by 4, 16, and 8 folds against *S. aureus* (ATCC 43300), respectively. It is in line with different previous similar studies in which plant extracts, demonstrated best results with different tested antibiotic (33–35).

All three tested extracts displayed moderate to high antibacterial activity on at least doubling of the 2 tested bacteria, with MIC values ranging from 7.8 µg/ml of Tetracycline and *T. rhynchoarpum*. Quart.-Dill. and A. Rich combination both tested *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 43300) at 62.5 µg/ml of Amoxicillin and *C. abyssinicum* Hochst. ex A. Rich combination against both tested bacterial strains and exhibited much more antibiotic modulating effect than some previous studies. The current antimicrobial test findings revealed that the interaction between the tested antibiotics and plant extract had a beneficial effect on the elimination of resistant bacteria and that combination boosted the efficiency of antimicrobial agents.

## 5.5. Conclusion and Recommendations

The current ethnomedicinal survey identified that the majority of the selected plant species were trees and herbs in growth habit. These plant species were claimed by as being utilized to treat different infections, including leishmaniasis, onchocerciasis, GI, wound, and skin infections. The major phytochemical classes of compounds with visible color changes and TLC spots were phenols and alkaloids. Flavonoids were remarkably exhibited with significant visible color change in *J. schimperiana* root, *C. macrostachyus* stem bark, *A. gumifera* stem bark *T. rhynchoarpum* root.

Alkaloid is the next most abundant class of compound present in *C. macrostachyus*, *C. hirsuta* and *C. abyssinicum*. All fractions of the extracts of *T. rhynchoarpum* root presented with the greatest activity against all selected strains with the lowest MICs; *J. schimperiana* root had the second highest activity against *P. aeruginosa*, *S. aureus*, and *E. coli*.

*Solanum nigrum*, *D. steudneri*, *T. dregaeha*, and *M. foetida* were shown to be ineffective against tested strains with MICs greater than 100 µg/ml. The combination of Tetracycline,

Amoxicillin, and Cloxacillin with *C. abyssinicum* Hochst. ex A. Rich, *C. hirsuta* Guill. and Perr, and *T. hynchocarpum* Quart.-Dill. & A. Rich crude extract) against *P. eruginosa* (ATCC 27853) and *S. aureus* (ATCC 43300) showed good and promising combinational effects.

All the findings were support scientific evidence for the usage of these plants as groundwork in traditional knowledge and point to a bright future for antibacterial drug research. Further pharmacological studies are required to be conducted using other microbial strains

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# 6

## **CHAPTER VI:**

### **BIOASSAY-GUIDED ISOLATION AND STRUCTURAL ELUCIDATION OF POTENTIAL ANTIMICROBIAL COMPOUNDS FROM *CRINUM ABYSSINICUM* ROOT BULB**

Part of the manuscript is submitted to frontiers in pharmacology (under review)

## Abstract

*Crinum abyssanicum* Hochst. ex A. Rich is a medicinal plant widely used traditionally for the treatment of wound infection, sexually transmitted diseases, cardiovascular diseases, hemorrhage, hepatitis, skin infection, gastro-intestinal infections, and cancer. Moreover, it needs substantial scientific evidence that substantiates its claim.

After fraction and antimicrobial test of root bulb extract of *Crinum abyssanicum*, the chloroform fraction which showed the lowest MIC against tested microbial strains were subjected to column chromatographic separation. Three compounds with relatively high antimicrobial efficacy were subjected to NMR analysis. It yielded hippadine (1), myrianthic acid (2), and lichexanthone (3). The structures of the isolated compounds were identified based on their NMR (1D and 2D) and MS spectral data analysis and comparison with reported literature data. This is the first report of the latter two compounds, including their classes, from the genus *Crinum*.

The crude extract, fractions, and isolated compounds were *in vitro* assayed against *Salmonella typhi*, *Salmonella enterics*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Shigella flexneri*, and *Leishmania donovani* strains. The test samples showed good activity, with the highest activity observed for the chloroform fraction with a MIC value of 1.9 µg/ml against *S. enteric*. Among the isolated pure compounds, compound Ca 40 and compound Ca 61 did not demonstrate satisfactory bioactivity with MIC >125. Ca 10 (Compound 1) had better activity, with MICs of 15.6 µg/mL against *S. enterics*, whereas the rest of the compounds showed moderate to weak activity against the tested strains. The observed anti-microbial activities of the extract, fractions and pure compounds could give insight into the potential of the medicinal plants as good sources for lead compounds in the development of anti-microbial drugs.

**Keywords:** Medicinal plants, anti-microbial, bio-guided, *Crinum abyssanicum*, compounds.

## 6.1. Introduction

Natural products serve as the foundation for the therapy of human and animal illnesses. A proven lead compound from a natural substance can make a substantial contribution to global economies and greatly impact the public health of the nation. Natural compounds are more likely to have a specific biological activity than "randomly" assembled synthetics. Despite its immense potential, only a small portion has ever been tested for bioactivity and chemical components (1–3). Plant products are valuable sources of therapeutic agents in most of today's drugs. Recent scientific developments, technological advances, and research trends indicate that natural products will be among the most important sources of new drugs in the future (4,5).

About half of all mortality in tropical areas is caused by infectious diseases. Numerous first-line antibacterial drugs have lost their efficacy as a result of the rise of multi-drug-resistant (MDR) strains of micro-organisms, which appears to be the main reason why treatments fail. The rise in microbial resistance to synthetic antibiotics has prompted researchers to explore the use of natural products with antibiotic actions from plants (6,7).

Human leishmaniasis is an infectious disease caused by different species and reported in many countries; it is considered as a major public health issue (8,9). Current treatments for the leishmanial species are unacceptable due to their route of administration, toxicity, expense, and resistance development. Several drug targets and lead molecules are being studied to develop new anti-leishmanial agents, but they are not promising, and the chemotherapy currently available for leishmaniasis is far from satisfactory (10,11). Today, there is a need for novel, efficient, and cost-effective medications to address microbial infections in the underdeveloped world. Finding "lead" molecules that could develop into useful medicines is an important first step (12–14).

*Crinum abyssanicum* is Hochst is a species of bulbous plant that belongs to the order Liliales and the family of Amaryllidaceae. It is believed to be native to the sunny areas of southwest Ethiopia and is recognized in the Oromo language (Afan Oromo) as "*Qullubbii waraabessaa*" and in Amharic language as "*Yejib shinkurt*" which can be translated as "Hyena's garlic. Traditionally, *C. abyssanicum* has been used as a remedy for wound healing, chancroid, hypertension, diabetes, hemorrhage, hepatitis, skin infection, internal parasites, and the

treatment of cancer. It contains different classes of phytochemical compounds, including flavonoids, alkaloids, glycosides, phenols, and saponins (15,16). Therefore, the objective of this experiment was to isolate and characterize a pure antimicrobial pure compound from *C. abyssinicum* Hochst. ex A. Rich root bulb. It might be a useful base for the development of affordable antimicrobial medicines.

## **6.2. Methods and Materials**

### **6.2.1, Plant Material**

*Crinum abyssinicum* Hochst.ex A. Arich root bulb was collected from Mattu district, Ilu Aba Bor Zone, Oromia regional state, southwest Ethiopia (8.2°N latitude and 35.5°E longitude, 15345 M above sea level). The plant material was identified by a botanist and stored at the Mattu University Herbarium with a voucher number of P03 (Fig 3.4)

### **6.2.2. Extraction and Isolation**

As illustrated under chapter 3 section 3.8, the air-dried plant material was powdered using a mortar and pestle to make it ready for extraction. Seven hundred grams of the air-dried and pulverized plant materials were extracted with chloroform and methanol at a ratio of 1:1 v/v three times for 24 hours each. The extracts were concentrated using a rotary evaporator at 40 °C to obtain 50 g (7.14%) of crude extracts. The crude extract was then suspended in water/Alcohol (1:9) and further partitioned based on previous similar studies (17–19). An antimicrobial activity test was performed on each fraction. The chloroform fraction exhibited good antimicrobial activity with the lowest MIC and MBC and a high Zone of Inhibition (ZI) and was subjected to column chromatography purification. Compound **1** (Ca 10) was obtained as 12 mg (0.05%) of a whitish solid with an R<sub>f</sub> value of 0.7 in a 7:3 n-hexane and ethyl acetate solvent system. Compounds **2** (Ca 36) was isolated as brown solid powder with an R<sub>f</sub> value of

0.52. Ca 40 was isolated with an R<sub>f</sub> value of 0.32 in a solvent system of 30% n-hexane and 70% ethyl acetate. Ca 75 (**3**) was isolated with an R<sub>f</sub> value of 0.45 after repeated eluting CC with 6 mg (0.03%) of white powder in a 20% n-hexane and 80% ethyl acetate solvent solution. Ca 81 was isolated at 1.2 mg with R<sub>f</sub> values of 0.7 as whitish powder at ethyl acetate 100%

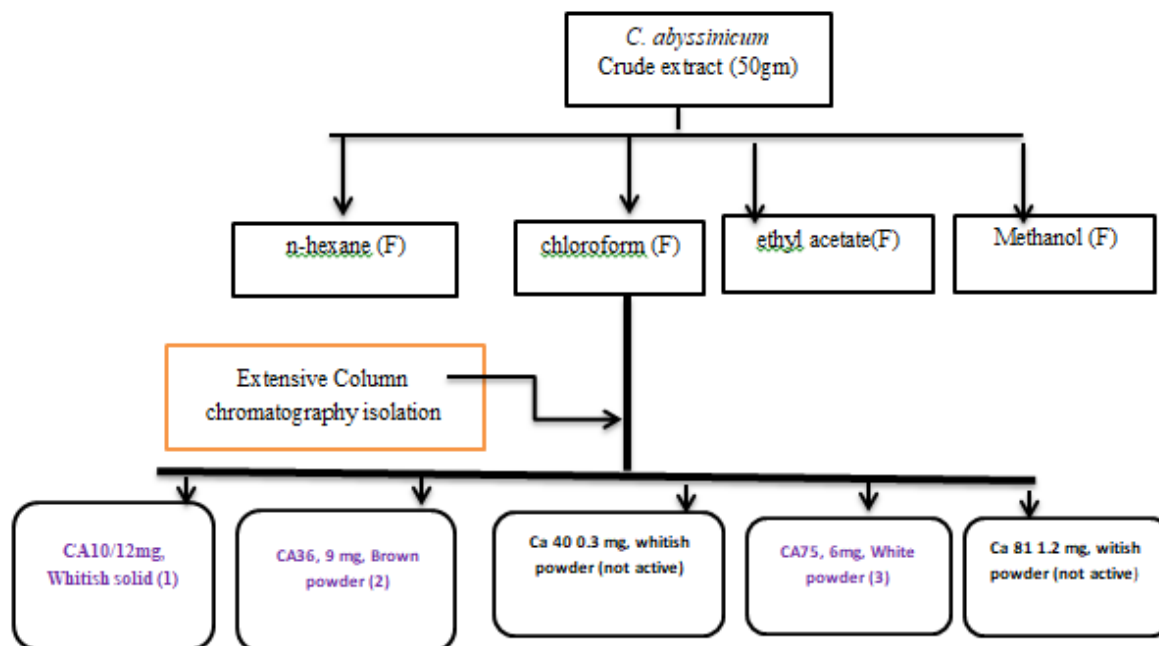


Figure 6.1: Flow of Bio-guided isolation the compounds from *C. abyssinicum*

### 6.2.3. *In-vitro* Antimicrobial Activity Tests

The antimicrobial values of the crude extract and fractions were determined using the broth microdilution method with 96-well microtiter plates. The reference antibiotics (ciprofloxacin and dicloxacillin) with a concentration range of 0.06-0.11 µg/mL were used as a positive control. After identifying the minimum inhibitory concentrations, the minimum bactericidal concentration MBC of the extracts was determined by sub culturing broths onto new agar plates. Serial nutrient agar plates were prepared and incubated at 37°C for 24 hours. After 24 hours on the agar plates, bacterial growth was assessed. The extract in which growths of the microbial strains were not visualized the MIC value were directly used as MBC. For the disc diffusion test, serial nutrient agar plates were prepared by pouring molten media into sterile petri dishes. The inoculums were then swabbed uniformly and allowed to dry for 5 minutes. Then, the filter paper discs (Whatman No. 1) measuring 6 mm were impregnated with the test

samples and placed on the surface of the medium. The Petri dishes were incubated at 37 °C for 24 h, and the diameter of the zone of inhibition was measured in mm. A similar procedure was adopted for the positive control antibiotics mentioned, and the corresponding zone of inhibition was compared accordingly. One percent of DMSO was used as a negative control, and the inhibition zones are divided into four:  $\leq 5$  mm diameter is categorized as weak, 6–10 mm diameter is moderate, 11–20 mm is strong, and  $\geq 21$  mm is very strong. An anti-promastigote assay was performed to determine the *in vitro* MIC of an already cultured *L. donovani* strain (Detail is mentioned under chapter III section 3.10. All tests were performed in triplicate and were determined based on previous similar studies (16,20,21).

### 6.3. Results

#### 6.3.1. Isolated Pure Compounds and Elucidated of the Structures

The chloroform fraction of *C. abyssinicum* Hochst.ex A. Rich, which showed superior antibacterial activity, was subjected to column chromatography on silica gel, and five compounds were isolated and three compounds were sent for structural elucidation (Fig. 6.2).

Compound 1 (CA-10) was isolated as a whitish solid with an  $R_f$  value of 0.7 in n- hexane: ethyl acetate (7:3). Its ESI-MS data analysis ( $m/z$  264  $[M+H]^+$ ) suggested the molecular formula of  $C_{16}H_9NO_3$  (Figure 6.3:). The  $^1H$  NMR spectrum showed seven proton signals in the aromatic region and one proton signal in the aliphatic region. The proton signal at  $\delta_H$  6.16 ppm (2H, s) accounts for the two methylenedioxy protons (H-12). The two singlet proton signals at  $\delta_H$  7.62 ppm (1H, s) and 7.96 ppm (1H, s) are indicative for aromatic protons in para positions (H-11 and H-8, respectively). Another proton signal at  $\delta_H$  7.89 (1H, d,  $J = 10$ ), 7.46 (1H, t,  $J = 10$ ), and 7.74 (1H, d,  $J = 10$ ) are ascribed to aromatic protons mutually coupling with each other and assigned to H-1, H-2, and H-3, respectively. This is also supported by the  $^1H$ - $^1H$  COSY spectra, which showed coupling for H-2 to both H-1 and H-3. Two additional proton signals at  $\delta_H$  6.89 (1H, d,  $J = 5$ ) and 8.03 ppm (1H, d,  $J = 5$ ) are attributed to the two ortho-coupled protons (H-4 and H-5, respectively) on the five-membered ring of the indole moiety. This is further confirmed by the  $^1H$ - $^1H$  COSY spectrum, in which H-4 and H-5 appeared as coupling partners. Moreover, a highly downfield-shifted aromatic proton signal at  $\delta_H$  8.03 ppm suggests the presence of an electronegative atom within an aromatic ring, implying that the ring is heterocyclic. The  $^{13}C$  NMR spectrum revealed the

presence of 16 carbon signals assignable to the methylene dioxy group ( $\delta_C$  102.7 ppm), aromatic carbons ( $\delta_C$  102.1, 108.4, 111.2, 117.0, 118.7, 122.8, 123.0, 123.9, 124.3, 128.8, 131.3, 132.0, 148.9, and 152.9 ppm), and carbonyl carbon of the amide group ( $\delta_C$  158.5 ppm). In corroboration with  $^1H$  NMR and  $^{13}C$  NMR spectral data, DEPT-135 spectra showed one negative signal ascribed to the methylene group flanked by two oxygen (C-12) and seven positive signals attributed to aromatic methines (C-1, C-2, C-3, C-4, C-5, C-8, and C-11). Chemical shift values were assigned based on HSQC data for protonated carbons and HMBC data for non-protonated carbons. In the  $^1H$ - $^1H$  COSY spectrum (Table 6.1)), H-1 is coupled to H-2, H-2 is coupled to H-1 and H-3, and H-4 is coupled to H-5, indicating that these hydrogens are adjacent to each other. In the  $^1H$ - $^1H$  COSY spectrum, H-8 and H-11 showed only cross peaks, which confirm that these two protons are not adjacent to any of the other protons.  $^1H$ - $^{13}C$  HMBC spectrum revealed correlations for H-1 to C-2 and C-11b via two bond coupling ( $^2J_{H-C}$ ), C-3, C-11a, and C-11c via the most common three bond coupling ( $^3J_{H-C}$ ), and C-3a and C-7a through four-bond coupling ( $^4J_{H-C}$ ). H-2 showed the correlation to C-1 and C-3 through two-bond coupling, C-11b and C-3a via three-bond coupling, and C-11a, C-11c, and C-4 via long-range (four-bond) coupling. H-3 showed the correlation to C-2 and C-3a through two bond couplings, C-1, C-4, and C-11c via three bond couplings, and C-11b and C-5 through four bond couplings. H-4 is correlated to C-5 and C-3a via two bond couplings, C-3 and C-11c through three bond couplings, and C-2 and C-11b via four bond couplings. H-5 is correlated to C-4 via two bond coupling, C-3a and C-11c via three bond coupling, and C-3, C-11b, and C-7a through four bond coupling; H-8 to C-7a via two bond coupling, C-11a via three bond coupling; and C-11, C-11b, and C-12 via four bond coupling. H-11 is correlated to C-11a through two bond couplings, C-11b through two bond couplings, and C-1 and C-8 via four bond couplings.  $^1H$ - $^{13}C$ -HMBC spectrum also revealed a correlation for H-12 to C-8 and C-11 via four bond coupling. Based on this evidence, compound 1 is identified as an alkaloid with a pyrrolophenanthridone nucleus (Lycorine type) named hippadine (( $C_{16}H_9NO_3$ )(Annex 2.1)).

Compound 2 was isolated as a solid powder with an  $R_f$  value of 0.52 in an n-hexane and ethyl acetate (1:4) solvent system. The  $^1H$ -NMR spectra (Table 6.2) showed proton signals assignable to methyl groups ( $\delta_H$  0.75, 0.78, 0.91, 0.99, 1.18, and 1.34 ppm),  $CH_2$  and  $CH$  groups in the aliphatic ring system ( $\delta_H$  1.25-2.60 ppm), protons attached to oxygenated

carbons ( $\delta_{\text{H}}$  3.27, 3.60, and 3.88 ppm), and an olefinic proton ( $\delta_{\text{H}}$  5.28 ppm).  $^{13}\text{C}$  NMR spectra also confirmed the presence of a pair of olefinic carbons at  $\delta_{\text{C}}$  128.78 (C-12) and 139.85 (C-13), one carboxylic acid carbon at  $\delta_{\text{C}}$  180.58 (C-28), and four oxygenated carbons at  $\delta_{\text{C}}$  66.66 (C-2), 78.81 (C-3), 73.12 (C-19), and 71.24 (C-23), in addition to the characteristic signals for the pentacyclitriterpene nucleus in the range of  $\delta_{\text{C}}$  16.54–54.67 ppm. The COSY spectra showed coupling between H-1 and H-2, H-5 and H-6, H-6 and H-7, H-11 and H-12, H-15 and H-16, H-20 and H-21, H-20 and H-30, and H-21 and H-22, which confirmed their placement on adjacent carbons. HMBC spectra revealed a correlation of H-23 to C-2, C-3, C-5, and C-24, supporting the placement of hydroxyl groups at the C-2 and C-3 positions. The HMBC spectrum also showed a correlation for H-24 to C-5 and C-23, which confirms the attachment of another hydroxyl group at C-23. The assignment of the remaining hydroxyl group at C-19 was also supported by the correlation observed for H-30 to C-19, C-20, C-21, C-22, and C-29 in the HMBC spectrum. Furthermore, the appearance of H-18 as a deshielded singlet signal in the  $^1\text{H}$  NMR spectrum is additional evidence for locating the remaining hydroxyl group at C-19. HMBC spectra revealed a correlation for the olefinic proton (H-12) to C-9, C-11, C-13, C-14, C-17, C-18, and C-27, indicating that the location of the double bond is between C-12 and C-13 of the usane nucleus. There is also evidence for locating the carboxyl group (COOH) at C-17 of the sane nucleus from the HMBC spectrum, which showed a correlation for H-15, H-18, and H-22 to carbonyl carbon (C-28) in common. HMBC data also supports the placement of methyl groups at C-4, C-8, C-10, C-14, C-19, and C-20 of the sane nucleus. Information obtained from the  $^1\text{H}$  NMR spectrum and the  $^{13}\text{C}$  NMR spectrum suggested tetrahydroxyursenoic acid. Based on this evidence and comparison with the literature, compound CA-36 is characterized as 2, 3, 19, 23-tetrahydroxyurs-12-en-28-oic acid ((myrianthic acid) (Table 6.2 and Annex 2.2)).

The third compound (**3**) was isolated as a whitish crystal, weighing 6 mg, in a solvent system of 0.5:9.5 n-hexane and ethyl acetate, with an  $R_{\text{f}}$  value of 0.45. Compound 3 (CA-75) was isolated as a whitish crystal, weighing 6 mg, in a solvent system of 20% n-hexane and 80% ethyl acetate, with an  $R_{\text{f}}$  value of 0.45. The  $^1\text{H}$  NMR spectrum (Table 6.3) of compound 3 showed one methyl singlet at  $\delta$  2.87 ppm (3H, -  $\text{CH}_3$ ), two singlets at 3.73 ppm (3H, -  $\text{OCH}_3$ ), and 3.77 ppm (3H, -  $\text{OCH}_3$ ), two aromatic singlets at  $\delta$  6.51 ppm (1H) and  $\delta$  6.82 ppm (1H), and two aromatic doublets at  $\delta$  6.57 ppm (1H) and  $\delta$  6.76 ppm (1H), corresponding to



two aromatic rings, and a singlet signal at  $\delta$  13.39 ppm (1H, OH), due to the presence of a hydroxyl group attached to the aromatic ring. Furthermore, this hydroxyl group is chelated to carbonyl oxygen via hydrogen bonding. The  $^{13}\text{C}$  NMR spectrum (Table 6.3) showed a total of 16 carbon signals assignable to  $\text{CH}_3$  groups ( $\delta\text{C}$  24.7 ppm),  $\text{OCH}_3$  groups ( $\delta\text{C}$  57.1 ppm), and aromatic carbons ( $\delta\text{C}$  93.6, 98.7, 100.4, 105.7, 114.3, 117.2, 144.7, 158.6, 161.0, 165.6, 165.7, 167.7, and 184.0 ppm). Information obtained from  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR suggested a xanthone nucleus bearing one methyl group, two methoxy groups, and one hydroxyl group, which was further corroborated by the 2D-NMR spectra, including COSY, HSQC, and HMBC data. The COSY spectrum revealed the absence of coupling partners, confirming that none of the protons are ortho to each other. HSQC spectra showed the correlation of H-2 to C-2, H-4 to C-4, H-5 to C-5, H-7 to C-7, H-14 to C-14, H-15 to C-15, and H-16 to C-16, corresponding to the assigned chemical shift values for protonated carbons. The HMBC spectrum revealed a correlation for H-2 to C-1 and C-3 ( $^2\text{J}_{\text{H-C}}$ ), C-4 and C-10 ( $3\text{J}_{\text{H-C}}$ ), and C-11 ( $4\text{J}_{\text{H-C}}$ ), whereas H-4 was correlated to C-1 ( $4\text{J}_{\text{H-C}}$ ), C-2 and C-10 ( $3\text{J}_{\text{H-C}}$ ), as well as C-3 and C-11 ( $2\text{J}_{\text{H-C}}$ ). H-5 was correlated to C-7 and C-13 ( $3\text{J}_{\text{H-C}}$ ), as well as C-12 ( $2\text{J}_{\text{H-C}}$ ), while H-7 showed a correlation to C-5 and C-13 ( $3\text{J}_{\text{H-C}}$ ), as well as C-12 ( $4\text{J}_{\text{H-C}}$ ). H-14 and H-16 showed a correlation to C-6 and C-3, respectively, via the three-bond ( $3\text{J}_{\text{H-C}}$ ), while H-15 was correlated to C-7 and C-13 ( $3\text{J}_{\text{H-C}}$ ), as well as C-8 ( $2\text{J}_{\text{H-C}}$ ). The HMBC spectra also showed a correlation for the OH proton between C-2 and C-10 ( $3\text{J}_{\text{H-C}}$ ), as well as C-1 ( $2\text{J}_{\text{H-C}}$ ). All these pieces of evidence confirmed that the structure of compound CA-75 is 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one ((Lichexanthone)(Table 6.3 and Annex 2.3))

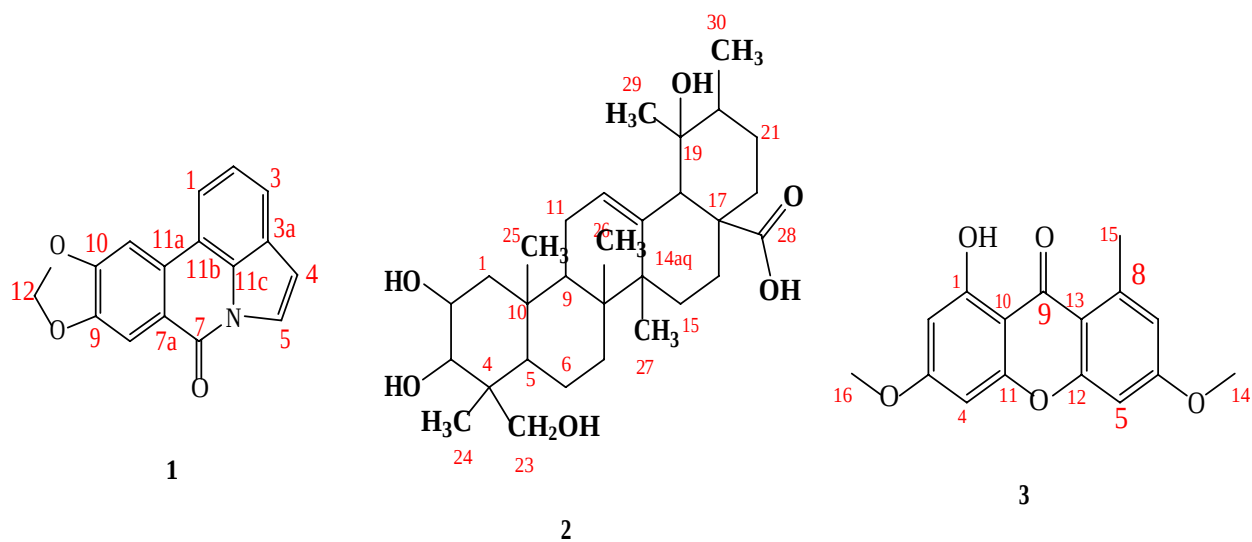


Figure 6.2: The structure of the isolated compounds

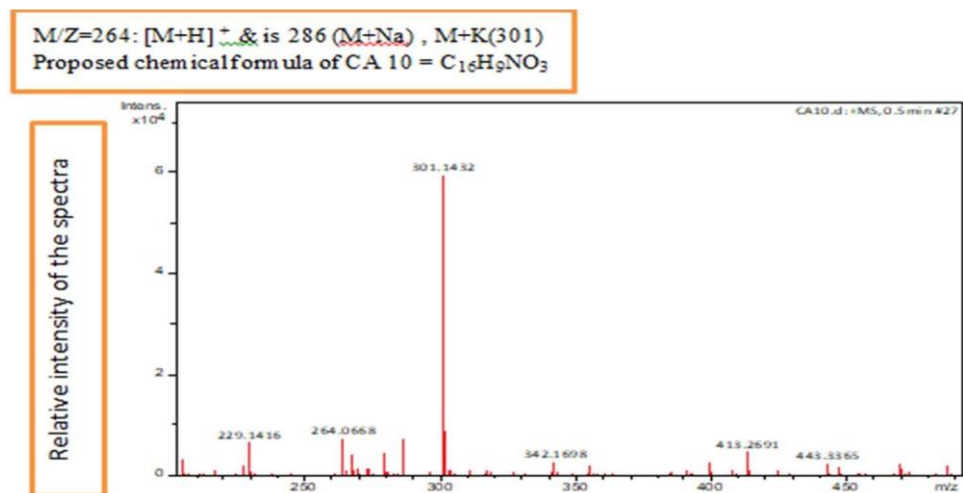


Figure 6.3: LC MS spectra of Compound 1(C a 10)

Table 6.1: <sup>1</sup>H NMR, <sup>13</sup>C NMR, COSY and HMBC spectral data of compound CA-10

| Position | $\delta^1\text{H}$  | $\delta^{13}\text{C}$ | COSY     | HMBC                                 |
|----------|---------------------|-----------------------|----------|--------------------------------------|
| 1        | 7.89 (1H, d, J= 10) | 118.7                 | H-2      | C-2,C-11b,C-3,C-11a,C-11c, C-3a,C-7a |
| 2        | 7.46 (1H, t, J=10)  | 124.3                 | H-1, H-3 | C-1,C-3,C-11b,C-3a,C-11a,C-11c,C-4   |
| 3        | 7.74 (1H, d, J=10)  | 123.0                 | H-2      | C-2,C-3a,C-1,C-4,C-11c,C-11b,C-5     |
| 3a       | ----                | 128.8                 | ----     | ----                                 |
| 4        | 6.89 (1H, d, J=5)   | 111.2                 | H-5      | C-5,C-3a,C-3,C-11c,C-2,C-11b         |
| 5        | 8.03 (1H, d, J=5)   | 123.9                 | H-4      | C-4,C-3a,C-11c,C-3,C-11b,C-7a        |
| 7        | ----                | 158.5                 | ----     | ----                                 |
| 7a       | ----                | 132.0                 | ----     | ----                                 |
| 8        | 7.96 (1H, s)        | 108.4                 | ----     | C-7a,C-11a,C-11,C-11b,C-12           |
| 9        | ----                | 148.9                 | ----     | ----                                 |
| 10       | ----                | 152.9                 | ----     | ----                                 |
| 11       | 7.62 (1H, s)        | 102.1                 | ----     | C-11a,C-11b,C-8,C-1                  |
| 11a      | ----                | 122.8                 | ----     | ----                                 |
| 11b      | ----                | 117.0                 | ----     | ----                                 |
| 11c      | ----                | 131.3                 | ----     | ----                                 |
| 12       | 6.16 (2H, s)        | 102.7                 | ----     | C-8,C-11                             |

Table 6.2. <sup>1</sup>H NMR, <sup>13</sup>C NMR COSY and HMBC spectral data of compound CA-36 compared

| CA-36    |                    |                       |            |                                               | Myrianthic acid [22]                |
|----------|--------------------|-----------------------|------------|-----------------------------------------------|-------------------------------------|
| Position | $\delta^1\text{H}$ | $\delta^{13}\text{C}$ | COSY       | HMBC ( $^2J$ , $^3J$ and $^4J_{\text{H-C}}$ ) | $\delta^{13}\text{C}$ (Pyridine-d5) |
| 1        | 1.37 (2H, m)       | 42.20                 | H-2        | C-5,C-8,C-10                                  | 41.38                               |
| 2        | 3.88 (1H, m)       | 66.66                 | H-1        | C-1,C-5,C-24,C-25                             | 64.90                               |
| 3        | 3.60 (1H, d, J=5)  | 78.81                 | ----       | C-1,C-2,C-5,C-24                              | 76.06                               |
| 4        | ----               | 43.65                 | ----       | ----                                          | 43.51                               |
| 5        | 1.62 (1H, m)       | 42.74                 | H-6        | C-2,C-3,C-7,C-9,C-10,C-23,C-24,C-25           | 46.79                               |
| 6        | 1.37 (2H, m)       | 18.77                 | H-5, H-7   | C-1,C-8,C-10, C-14                            | 17.74                               |
| 7        | 1.25, 1.63 (2H, m) | 33.43                 | H-6        | C-4,C-5,C-8,C-9,C-10,C-14, C-26               | 32.51                               |
| 8        | ----               | 40.82                 | ----       | ----                                          | 39.55                               |
| 9        | 1.87 (1H, m)       | 48.05                 | ----       | C-1,C-5,C-10,C-11,C-14,C-25,C-26,C-27         | 47.05                               |
| 10       | ----               | 38.76                 | ----       | ----                                          | 37.85                               |
| 11       | 1.36,1.97 (2H, m)  | 24.77                 | H-12       | C-1,C-8,C-9,C-10,C-12,C-13,C-14               | 23.37                               |
| 12       | 5.25 (1H, t, J=5)  | 128.78                | H-11       | C-9,C-11,C-13,C-14,C-17,C-18,C-27             | 126.9                               |
| 13       | ----               | 139.85                | ----       | ----                                          | 138.84                              |
| 14       | ----               | 42.07                 | ----       | ----                                          | 41.33                               |
| 15       | 1.72,2.60 (2H, m)  | 27.06                 | H-16       | C-9,C-14,C-17,C-18, C-28                      | 28.19                               |
| 16       | 1.51 (2H, m)       | 26.39                 | H-15       | C-14, C-27                                    | 25.35                               |
| 17       | ----               | 48.05                 | ----       | ----                                          | 47.05                               |
| 18       | 2.50 (1H, s)       | 54.67                 | ----       | C-12,C-13,C-14,C-16,C-17,C-19,C-20,C-28       | 53.36                               |
| 19       | ----               | 73.12                 | ----       | ----                                          | 71.81                               |
| 20       | 1.27 (1H, m)       | 42.44                 | H-21,H-30  | C-17,C-21,C-22,C-29                           | 41.57                               |
| 21       | 1.72,2.60 (2H, m)  | 27.06                 | H-20, H-22 | C-17,C-18,C-20,C-28                           | 26.12                               |
| 22       | 1.60,1.71 (2H, m)  | 38.66                 | H-21       | C-16,C-17,C-18,C-20,C-28                      | 37.47                               |
| 23       | 3.27 (2H, s)       | 71.24                 | ----       | C-2,C-3,C-5,C-24                              | 69.34                               |
| 24       | 1.18 (3H, s)       | 17.12                 | ----       | C-5,C-23                                      | 16.65                               |
| 25       | 0.75 (3H, s)       | 17.40                 | ----       | C-1,C-4,C-5,C-9                               | 16.85                               |
| 26       | 0.78 (3H, s)       | 17.54                 | ----       | C-7,C-9,C-14                                  | 17.05                               |
| 27       | 1.34 (3H, s)       | 24.44                 | ----       | C-8,C-9,C-12,C-13,C-14,C-26                   | 24.27                               |
| 28       | ----               | 180.58                | ----       | ----                                          | 179.13                              |
| 29       | 0.99 (3H, s)       | 27.03                 | ----       | C-17, C-20,C-30                               | 26.59                               |
| 30       | 0.91 (3H, d, J=10) | 16.54                 | H-20       | C-19,C-20,C-21,C-22,C-29                      | 16.48                               |

Table 6.3: <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HMBC spectral data of compound CA-75 compared to reported data of Lichexanthone

| CA-75    |                  |                   |                          | Lichexanthone [23]                              |                                                  |
|----------|------------------|-------------------|--------------------------|-------------------------------------------------|--------------------------------------------------|
| Position | δ <sup>1</sup> H | δ <sup>13</sup> C | HMBC                     | δ <sup>1</sup> H<br>(400MHz CDCl <sub>3</sub> ) | δ <sup>13</sup> C<br>(100MHz CDCl <sub>3</sub> ) |
| 1        | ----             | 165.7             | ----                     | ----                                            | 163.8                                            |
| 2        | 6.51 (1H, s)     | 98.7              | C-1,C-3, C-4, C-10,C-11  | 6.29                                            | 96.8                                             |
| 3        | ----             | 167.7             | ----                     | ----                                            | 165.9                                            |
| 4        | 6.57 (1H,d, J=5) | 93.6              | C-1, C-2, C-3, C-10,C-11 | 6.32                                            | 92.1                                             |
| 5        | 6.76 (1H, d,J=5) | 100.4             | C-7,C-12, C-13           | 6.65                                            | 98.5                                             |
| 6        | ----             | 165.6             | ----                     | ----                                            | 163.7                                            |
| 7        | 6.82 (1H, s)     | 117.2             | C-5, C-12, C-13          | 6.67                                            | 115.4                                            |
| 8        | ----             | 144.7             | ----                     | ----                                            | 143.8                                            |
| 9        | ----             | 184.0             | ----                     | ----                                            | 182.7                                            |
| 10       | ----             | 105.7             | ----                     | ----                                            | 104.3                                            |
| 11       | ----             | 158.6             | ----                     | ----                                            | 157.0                                            |
| 12       | ----             | 161.0             | ----                     | ----                                            | 159.5                                            |
| 13       | ----             | 114.3             | ----                     | ----                                            | 113.0                                            |
| 14       | 3.75 (3H, s)     | 57.1              | C-6                      | 3.89                                            | 55.7                                             |
| 15       | 2.87 (3H, s)     | 24.7              | C-7, C-8, C-13           | 2.84                                            | 23.4                                             |
| 16       | 3.75 (3H, s)     | 57.1              | C-3                      | 3.86                                            | 55.6                                             |
| 1-OH     | 13.88 (1H, s)    | ----              | C-1, C-2, C-10           | 13.39                                           | ----                                             |

### 3.2. Antimicrobial Activity

The crude extract of the tested plant (*C. abyssinicum*) exhibited MIC of 7.8 µg/mL against *S. typhi* and 15.6 µg/ml against *S. enterics* and *Sh. flexineri*. The chloroform portion had the highest activity, with a MIC of 1.9 µg/ml against *S. enterics* and 3.9 µg/ml against *S. styph* and *S. flexneri*. Compound 1 presented modest activity, with MICs of 15.6 µg/ml and 31.25 µg/ml against *S. enterics* and *S. styph*, respectively, and a zone of inhibition (ZI) of 20 mm and 19 mm against *E. aerogenes* and *S. flexineri*, respectively (Table 6.4). The lowest inhibitory concentration against *L. donovani* was presented only with the methanol fraction and the third isolated compound (3). The methanol fraction and the third isolated compound (3) of *C. abyssinicum* Hochst.ex A. Rich root bulb presented MICs of 31.25 µg/mL and 15.6 µg/ml against tested *L. donovani*, respectively.

Table 6.4: MIC of crude and different fractions of *Crinum abyssinicum* Hochst.ex A. Rich against selected bacterial strains

| Extranets                              | In-vitro measurements | microbial trains |             |       |       |       |       |
|----------------------------------------|-----------------------|------------------|-------------|-------|-------|-------|-------|
|                                        |                       | SE               | ST          | SF    | EBA   | EBC   | LD    |
| Crude extract                          | MIC (µg/ml)           | 15.6             | 7.8         | 15.6  | 31.25 | 31.25 |       |
|                                        | MBC (µg/ml)           | 31.25            | 31.25       | 31.25 | 62.5  | 62.5  | 125   |
|                                        | ZI (mm)               | 19               | 19          | 23    | 23    | 20    |       |
| n-hexane fraction                      | MIC (µg/ml)           | 31.25            | 62.5        | 62.5  | 62.5  | 62.5  | 500   |
|                                        | MBC (µg/ml)           | 62.5             | 125         | 125   | 125   | 125   |       |
|                                        | ZI (mm)               | 18               | 17          | 20    | 21    | 17    | 500   |
| DichlorométhanDichloromethane fraction | MIC (µg/ml)           | 1.9              | 3.9         | 3.9   | 7.8   | 7.8   |       |
|                                        | MBC (µg/ml)           | 7.8              | 7.8         | 7.8   | 15.6  | 15.6  | 250   |
|                                        | ZI (mm)               | 19               | 18          | 21    | 22    | 19    |       |
| Ethyl acetate                          | MIC (µg/ml)           | 31.25            | 15.6        | 15.6  | 31.25 | 62.5  | 125   |
|                                        | MBC (µg/ml)           | 31.25            | 31.25       | 31.25 | 62.5  | 125   |       |
|                                        | ZI (mm)               | 17               | 18          | 19    | 21    | 17    | 31.25 |
| Methanol residue                       | MIC (µg/ml)           | 31.25            | 31.25       | 31.25 | 62.5  | 62.5  |       |
|                                        | MBC (µg/ml)           | 62.5             | 62.5        | 62.5  | 62.5  | 62.5  |       |
|                                        | ZI (mm)               | 17               | 17          | 19    | 20    | 17    |       |
| Ca 10 Compounds                        | MIC (µg/ml)           | 15.6             | 15.6        | 15.6  | 31.25 | 62.5  | 125   |
|                                        | MBC (µg/ml)           | 31.25            | 31.25       | 31.25 | 62.5  | 62.5  |       |
|                                        | ZI (mm)               | 18               | 18          | 19    | 20    | 18    |       |
| Ca 40<br>Ca36 Compound                 | MIC                   | 125              | 31.25       | 31.25 | 62.5  | 62.5  | 62.5  |
|                                        | MIC (µg/ml)           | 31.25            | 62.5        | 62.5  | 62.5  | 62.5  |       |
|                                        | MBC (µg/ml)           | 62.5             | 18          | 19    | 20    | 17    |       |
| Ca 75 Compound                         | ZI (mm)               | 17               | 62.5        | 62.5  | 125   | 125   | 15.6  |
|                                        | MIC (µg/ml)           | 62.5             | 125         | 125   | 125   | 125   |       |
|                                        | MBC (µg/ml)           | 125              | 18          | 20    | 19    | 17    |       |
| Ca 81                                  | ZI (mm)               | 17               | <0.06/<0.06 | 0.06  | <0.06 | 0.11  |       |
|                                        | MIC                   | >500             | 20          | 21    | 23    | 22    |       |
|                                        |                       |                  |             |       |       |       |       |
| Ciprofloxacin                          | MIC/MBC               | 0.11/0.11        | 0.11        | <0.06 | 0.11  | 0.11  |       |
|                                        | ZI                    | 20               | 20          | 22    | 23    | 22    |       |
| Dicloxacillin                          | MIC/MBC               | <0.06            |             |       |       |       | 0.06  |

KEY: - SE: *Salmonella* Enterics NR13555, ST: *Salmonella* styphcbc, EBA: *Enterobacter aerogenes*, EBC: *Enterobacter cloacae*, SF: *Shigella flexneri*, LD: *Leishmania* Donovan. MIC, Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, ZI: Zone of inhibition

#### 6.4. Discussion

The air-dried root bulb of *C. abyssinicum* was extracted with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:1) by cold percolation at room temperature. In our current study, the extract was further partitioned successively with n-hexane and dichloromethane. The dichloromethane fraction was then subjected to silica gel column chromatography following its superior antibacterial activity and afforded three compounds. Based on the evidence generated from NMR spectra, compound 1 is identified as an alkaloid with a pyrrolophenanthridone nucleus (Lycorine type) named hippadine (C<sub>16</sub>H<sub>9</sub>NO<sub>3</sub>). This compound was previously isolated from the same plant species and from the *Crinum macowanii* species (22,23).

The evidence generated from NMR and available literature indicate that compound 2 is characterized as 2,3,19,23-tetrahydroxyurs-12-en-28-oic acid (myrianthic acid). This compound was previously reported from the leaves of *Campsis grandiflora* but is new from *C. abyssinicum* (24). All these pieces of evidence NMR and previous studies confirmed that the structure of compound 3 is 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone). This compound was previously reported from *Vismia baccifera* but is also new from this plant (25).

As illustrated in Table 4, the *in vitro* MIC of the antibacterial and anti-leishmanial activity of different fractions and pure compounds of the root bulb of *C. abyssinicum* has shown promising activity against tested microorganisms. The crude extract was presented with MICs of 7.8 µg/mL against *S. typhi* and 15.6 µg/mL against *S. enterics* and *Sh. flexneri*. The DCM portion had the highest activity, with a MIC of 1.9 µg/mL against *S. enterics* and 3.9 µg/mL against *S. styph* and *S. flexneri*. When compared to crude extract, the chloroform fractions' activities were elevated. It could be because most active groups of chemicals are extracted in moderately polar solvents. This finding contrasts with other comparable studies, which found that the crude extract is more active in various studies due to the synergistic impact [11, 19]. Similar to our earlier studies by Dubale S. et al. (2023), Waltrich K. et al. (2015), and Shahla M. et al. (2001), the n-hexane fraction was found to be only modestly active against all examined strains, while the DCM fraction was found to be the most active [14,20,21].

Compound 1 presented modest activity, with MICs of 15.6 µg/mL and 31.25 µg/mL against *S. enterics*, *S. styph*, and *S. flexineri*, respectively, and a zone of inhibition (ZI) of 20 mm and 19 mm against *E. aerogenes* and *S. flexineri*, respectively. The lowest inhibitory concentration against *L. donovani* was presented only with the methanol fraction and the third isolated compound. The methanol fraction and third isolated compound 3 of *C. abyssinicum* root bulb presented MICs of 31.25 µg/mL and 15.6 µg/mL against tested *L. donovani*, respectively. This may be due to the high polarity of the fraction, as reported in previous similar studies (20, 22).

All the fractions of the plant extract and isolated compounds exhibited moderate antimicrobial activity. It less effective against all tested strains when compared with the reference antibiotics ciprofloxacin, dicloxacillin, and amphotericin B. Finally, non parametric

## 6.5. Conclusion

*Crinum abyssinicum* is a plant species that has traditionally been used to treat a range of ailments in Ethiopia. The chloroform fraction afforded three compounds, viz., hippadine (1), myrianthnic acid (2), and lichexanthone (3).

The efficacy of chloroform portion had the highest activity, with a MIC of 1.9 µg/ml against *S. enterics* and 3.9 g/ml against *S. styph* and *S. flexneri*, and it contrasts with other comparable studies. Only the methanol fraction and lichexanthone (3) had the lowest inhibitory concentrations against *L. donovani*.

As an alternative, isolated compounds from *C. absnicum* could be a potential answer for the development of an active antimicrobial. Further biological activities such as anti-viral, anti-cancer, anti-inflammatory, anti-oxidant, and anti-diabetic tests must be considered, and modifying the structure of the compounds can have beneficial impacts.

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# 7

## **CHAPTER VII: TRADITIONAL MEDICINE REGULATORY FRAMEWORK AND LEGAL BASIS IN ETHIOPIA: A CRITICAL EVALUATION OF CHALLENGES AND OPPORTUNITIES FOR POLICY IMPLEMENTATION**

Part of this manuscript is submitted to the BMC journal for publication (under review)

## **Abstract**

**Background:** The efficacy, quality and safety of traditional medicine used by the Ethiopian population may be questionable. Strict regulation is crucial to protect the public from unsafe and ineffectual traditional medicine.

**Objective:** The primary aim of the study was to assess the legal framework and its practical implementation status for the regulation of traditional medicine in Ethiopia.

**Methods:** institution-based cross-sectional study was conducted from November 2021 to March 2022. Archival review, literature search, and were applied for data collection. Data were analyzed using IBM® SPSS version 25.0, USA. The result was presented using descriptive and inferential statistics at <0.05 significance level and 95% confidence interval.

**Results:** Ethiopia is regulating traditional medicines based on the Medicine Policy of 1993 and the Food and Drug Authority of Ethiopia's Proclamation No. 1112/2019. About 70.2% of the federal regulatory officers and 41.7% of the regional regulatory officers responded that a traditional medicine regulatory activity in Ethiopia is weak. Barriers include poor governance, resource limitations, lack of stakeholder cooperation; have had the most significant associations with weak regulatory implementation.

**Conclusion:** The current Ethiopian traditional medicine regulation framework is inadequate to ensure quality, safety, efficacy, and rational use of traditional medicine. Enhancing the framework is crucial for a more effective and accessible implementation of regulation.

**Key words:** Ethiopia, Traditional Medicine, proclamation, legal- framework, regulation, safety, quality

## 7.1. Introduction

It is estimated that one-third of the world's populations, particularly in developing countries, depend on traditional medicine (TM) for the fulfillment of their primary health care needs (1,2). The majority of countries lack regulations over TM products and practices (3,4). As a result, the safety, quality, and efficacy of TM as well as the access to better healthcare is hampered (5,6). Since the Alma-Ata declaration, several nations have made important efforts for integrative methods that allow the provision of safe, quality, and cost-effective, TM products and services. However, the absence of a regulatory framework for the TM system and weak regulation implementation can have negative effects on human and animal health. But most of the TM users believe that TM is completely safe, in contrast to scientific evidence (2,6,7).

Providing the best product for citizens is the responsibility of every government. This can be accomplished by developing genuine policy, and implementing it effectively (8,9). Most countries have already developed their legal framework (10,11). The pharmaceutical industry currently invests a significant amount of money to ensure the safety and efficacy of modern medicines, which must meet criteria such as bioavailability, toxicity, safety, clinical data, and others. However, TM practices and products receive little attention (12,13). Like other African countries, most Ethiopian populations rely on traditional medicine to treat a variety of ailments. In Ethiopia, TMPs utilize natural compounds made up of plants, animals, and minerals together with spiritual healing and bone setting (1,8,14,15).

Ethiopia established the legal acknowledgement of TM in "Proclamation 27/1942. Since the 1970s, the country's health strategy has been more concerned with disease prevention and health promotion, particularly the growth of rural health facilities (3,16,17). The office for the coordination of TM was established in November 1979, but there was a lack of direction and resources (finance, and human) for forwarding activities. TM practice and product usage remain uncontrolled, and their safety and therapeutic usefulness are not always assured (18–21). This can be accomplished through legitimate policy and regulatory frameworks. The contribution of a legal framework is crucial since it establishes the mechanism for the formation and operation of a lawful TM regulatory system (22,23).

The level of development of the legal basis, the development of the health sector, the availability of trained human resources, setups, the size and sophistication of the regulatory

authority, political concern, and other related factors may influence the activity of regulatory functions (24–26). Therefore, the objectives of this study were to assess the current legal basis and practical implementation of TM regulation in Ethiopia. The evidence generated from the study could be useful for policymakers, academicians, researchers', traditional medicinal practitioners, and the end users of TM at large. The study facilitates identifying the gaps, weaknesses, challenges, and opportunities of implementation of TM practices and product regulation in Ethiopia.

## **7.2. Methods and Materials**

### **7.2.1. Study Design and Period**

An institutional-based cross-sectional study design was conducted from November 2021 to March 2022.

### **7.2.2. Study Area**

The study was conducted in three purposively selected regions, namely Oromia regional state, Addis Ababa city, and SNNP regional state.

Population, Inclusion Exclusion Criteria and Sample Size Determination were stated under chapter III section 3.4 and Figure 7.1: also presented the schematic presentation of sampling technique

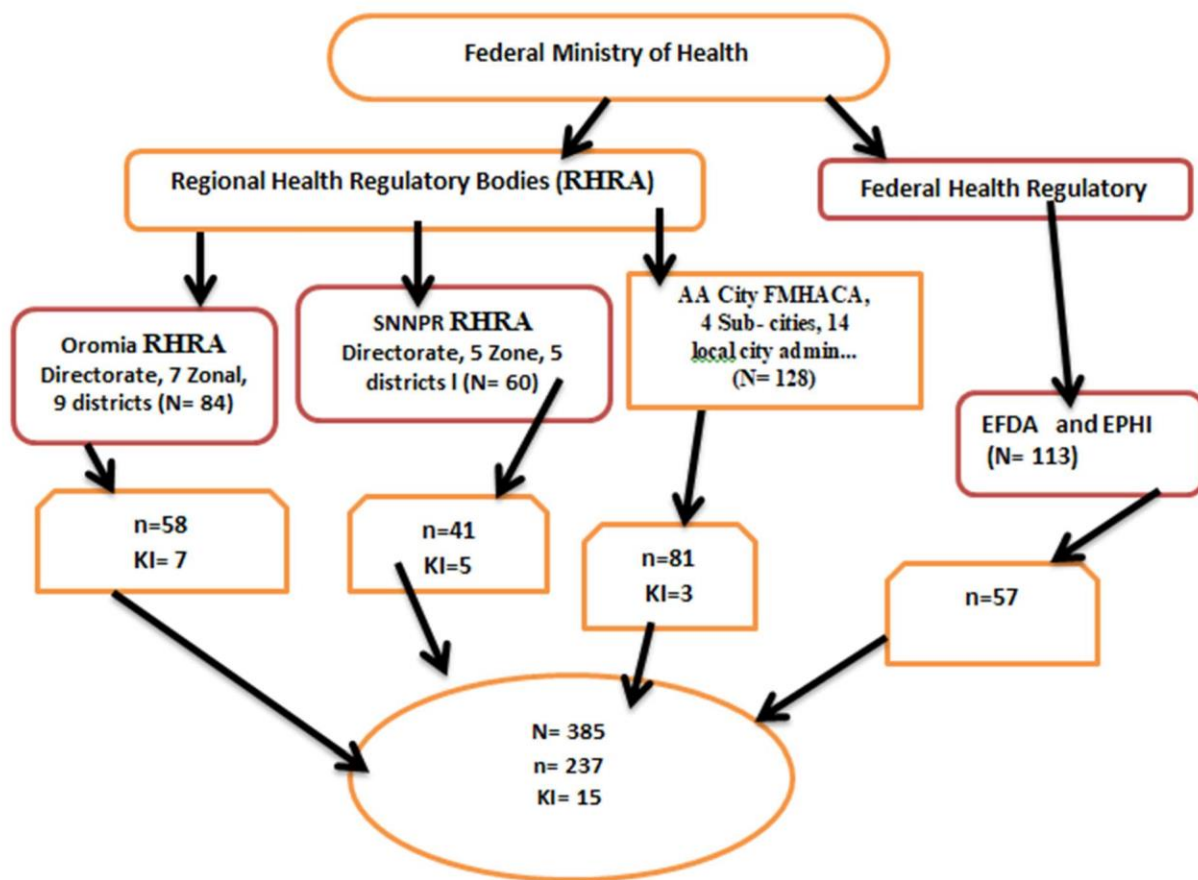


Figure 7.1: Schematic presentation of sampling technique utilized to select sample frame

### 7.2.3. Data Collection Procedure

The data was collected using a combination of an archive assessment of Ethiopia's legislative basis, in-depth interviews, self-administrative questionnaires', and literature searches (Chapter III section 3.4) .

### 7.2.4. Data Analysis and Presentation

The findings were summarized and presented descriptively using tables and figures. The organized data was entered into Epi-Data Manager Version 4.6. Then it was exported and analyzed using IBM® Statistical Package for the Social Sciences (SPSS) version 25.0, USA. Bivariate and multivariate analyses were used to identify major challenges associated with the weak performance of TM regulatory offices on the implementation of TM regulation at 95% confidence levels and a p-value <0.05.



## **7.3. Results**

### **7.3.1. Archival Review and Literature Search**

Traditional Medicine regulation in Ethiopia is carried out in conjunction with modern medicine under proclamation No. 1112/2019. The Ethiopian Food and Drug Authority, MoH TM desk, and RHR regulatory offices, are currently implementing the TM regulation collaboratively. Traditional medicine is defined as ‘medicine’ under proclamation article 2/9, whereas the definitions of TM practice and practitioner are not addressed in this proclamation. So far, there is no officially registered TM product by the federal regulatory authority, although some TM preparations are available on the market. The data obtained from the Oromia regional regulatory authority showed that about 200 TMP were registered before the time of data collection but no officially registered TMP at Federal level. No TM education system in Ethiopia. The code of ethics for TMP, the TM national expert committee, TM local production, national monographs for TM, the TM national pharmacopoeia, the NEML of TM products, the national plan for integrating TM into the national health service delivery, and related information are not available in Ethiopia's TM policy. The draft preparation of the TM policy has been completed, but it has not yet been endorsed (Table 7.1).

Table 7.1: List of some WHO member countries reviewed for performance of TM regulation status

| Countries    | NP and strategic included TM |   | Exclusive TM policy | Legal framework for TM | Regulation framework | Code of ethics for TMP | Policy implementation strategic | ITM programme in Ministry of Health | National expert committee for TM | TM research institutes | Local production of TM products | Regulation systems for HM | Registration systems for HM | Monographs for he HM | Pharmacopoeia for HM | NEML of TM products | Institutionalized training programmers | Collaboration between TMPs and CHPs | Plan for integrating TM into the MM | Health insurance for TM | Public and/or government fund for TM | Country Total Mark for Y only in %. |
|--------------|------------------------------|---|---------------------|------------------------|----------------------|------------------------|---------------------------------|-------------------------------------|----------------------------------|------------------------|---------------------------------|---------------------------|-----------------------------|----------------------|----------------------|---------------------|----------------------------------------|-------------------------------------|-------------------------------------|-------------------------|--------------------------------------|-------------------------------------|
| Benin        | U                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | U                    | U                   | Y                                      | U                                   | Y                                   | A                       | Y                                    | 72.7                                |
| Cameroon     | Y                            | Y | Y                   | Y                      | Y                    | U                      | Y                               | U                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | U                    | Y                   | U                                      | U                                   | U                                   | U                       | Y                                    | 63.6                                |
| Ethiopia     | Y                            | Y | Y                   | A                      | U                    | Y                      | Y                               | U                                   | Y                                | U                      | U                               | Y                         | U                           | U                    | U                    | U                   | U                                      | Y                                   | U                                   | U                       | Y                                    | 50                                  |
| Ghana        | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | Y                   | Y                                      | U                                   | Y                                   | U                       | Y                                    | 90.9                                |
| Kenya        | Y                            | U | U                   | A                      | U                    | U                      | Y                               | U                                   | U                                | U                      | U                               | U                         | U                           | U                    | U                    | U                   | U                                      | U                                   | U                                   | U                       | U                                    | 13.6                                |
| Madagascar   | Y                            | Y | Y                   | Y                      | U                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | Y                   | Y                                      | U                                   | U                                   | U                       | Y                                    | 77.3                                |
| Mali         | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | U                         | Y                           | Y                    | U                    | Y                   | Y                                      | Y                                   | Y                                   | Y                       | Y                                    | 90.9                                |
| Nigeria      | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | U                   | Y                                      | Y                                   | U                                   | U                       | Y                                    | 86.4                                |
| Senegal      | U                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | U                           | Y                    | U                    | U                   | Y                                      | Y                                   | U                                   | U                       | U                                    | 59.1                                |
| South Africa | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | U                   | Y                                      | Y                                   | U                                   | Y                       | Y                                    | 90.9                                |
| Uganda       | U                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | U                   | Y                                      | Y                                   | U                                   | U                       | Y                                    | 77.3                                |
| Tanzania     | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | U                           | U                    | U                    | U                   | Y                                      | Y                                   | U                                   | U                       | Y                                    | 68.2                                |
| Zimbabwe     | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | U                                   | Y                                | Y                      | U                               | U                         | Y                           | U                    | U                    | U                   | U                                      | Y                                   | U                                   | U                       | Y                                    | 59.1                                |
| China        | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | Y                   | Y                                      | Y                                   | Y                                   | Y                       | Y                                    | 100                                 |
| India        | Y                            | Y | Y                   | Y                      | Y                    | Y                      | Y                               | Y                                   | Y                                | Y                      | Y                               | Y                         | Y                           | Y                    | Y                    | Y                   | Y                                      | Y                                   | Y                                   | Y                       | Y                                    | 100                                 |

Note: - U= Information not Available, A= Absent, Y= Present

### 7.3.2. Socio-demographic characteristics of respondents

Among the 237 regulatory professionals who participated in the survey, the majority of respondents professions were pharmacists (75.5%), males (66.2%), and first degree in education (83.1%). The average respondent's experience as a regulatory officer was 4.2 years (Table 7.2).

Table 7.2: Demographic characteristics of regulatory personnel during the study period, 2022 (n = 237)

| Parameters                       |                  |                      | Freq. (n) | Percent (%) |
|----------------------------------|------------------|----------------------|-----------|-------------|
| Gender                           |                  | Male                 | 179       | 75.5        |
|                                  |                  | Female               | 58        | 24.5        |
| Profession                       |                  | Pharmacy             | 157       | 66.2        |
|                                  |                  | Environmental Health | 24        | 10.1        |
|                                  |                  | Health Officer       | 37        | 15.6        |
|                                  |                  | Nursing              | 15        | 6.3         |
|                                  |                  | Medical Laboratory   | 4         | 1.7         |
|                                  |                  |                      |           |             |
| Education level                  |                  | Diploma              | 4         | 1.7         |
|                                  |                  | Degree               | 197       | 83.1        |
|                                  |                  | MSc                  | 36        | 15.2        |
| Level of regulation offices      | Federal          | EFDA & EPHI          | 55        | 24.1        |
|                                  |                  |                      | 2         |             |
|                                  | Oromia region    | Oromia region        | 15        | 6.3         |
|                                  |                  | zone/town offices    | 43        | 18.1        |
|                                  | SNNPR            | regional office      | 16        | 6.8         |
|                                  |                  | zone/town offices    | 25        | 10.5        |
|                                  | Addis Ababa city | Addis Ababa city     | 11        | 4.6         |
|                                  |                  | Sub-cities offices   | 13        | 5.5         |
|                                  |                  | Local city offices   | 57        | 24.1        |
| Respondent regulation experience | ≤ 4 year         | 139                  | 58.6      |             |
|                                  | > 4 years        | 98                   | 41.4      |             |

SNNPR: South Nation and nationality people region

### 7.3.3. Availability of TM Product Regulation Systems and Frameworks

Among the 57 regulatory personnel from the EFDA, 46 (80.7%) of them responded that TM products are regulated as an exclusive category. Regarding pharmacovigilance (PV), most of the 57 (87.7%) federal-level participants responded that the EFDA reporting system is only available for conventional pharmaceuticals and does not include TM products. The majority of the study participants noticed the absence of a regulatory system that was enforced based on the claims of TMs products in Ethiopia ((Table 7.3).

Table 7.3: Availability of TM product regulatory systems, regulatory categories and their claims (n = 57)

| Variables                                               |                                                   | n  | %     |
|---------------------------------------------------------|---------------------------------------------------|----|-------|
| Regulation system for TM products                       | Yes                                               | 57 | 100   |
|                                                         | No                                                | 0  | 0.0   |
| TM regulation status                                    | Separate regulations                              | 46 | 80.7  |
|                                                         | It is not clearly known                           | 11 | 19.3  |
| TM registration system                                  | Yes                                               | 51 | 89.5  |
|                                                         | No                                                | 6  | 10.5  |
| TM product sold with any claim                          | Yes                                               | 57 | 100.0 |
|                                                         | No                                                | 0  | 0.0   |
| PV reporting system exist for TM                        | Yes                                               | 7  | 12.3  |
|                                                         | No                                                | 50 | 87.7  |
| TM product safety requirements                          | Exclusive safety requirements                     | 50 | 87.7  |
|                                                         | Traditional use without harmful effects           | 47 | 82.5  |
|                                                         | Reference to scientific research                  | 44 | 77.2  |
|                                                         | Safety requirements are not officially published  | 17 | 29.8  |
| TM GMP requirements                                     | Exclusive GMP regulations for TM                  | 42 | 73.7  |
|                                                         | GMP requirements are not officially published     | 15 | 26.3  |
| Mechanism used to ensure compliance of GMP requirements | Periodic inspections by authority                 | 36 | 63.2  |
|                                                         | Manufacturers submit samples of HM product        | 38 | 66.7  |
|                                                         | Manufacturers assign their responsible person     | 14 | 24.6  |
|                                                         | Assuring method GMP for compliances not published | 16 | 28.1  |
| TM regulatory status                                    | As self-medication                                | 40 | 70.2  |
|                                                         | As dietary supplement                             | 14 | 26.5  |
|                                                         | As none prescription medicine                     | 2  | 3.2   |
| TM claims based on regulatory status                    | No TM claimed based regulation status             | 54 | 94.7  |
|                                                         | Nutrient content claim but not regulated by law   | 3  | 5.3   |
|                                                         | Medical claims but not regulated by law           | 0  | 0     |

GMP: Good manufacturing practice

#### 7.3.4. Functionality of TM Products Regulatory Systems at The Federal Level

Among the 57 federal level regulatory officers, the majority (87.7%) of them agreed on the creation of functionality of TM manufacturers. About 77.2% of regulatory personnel agreed on the regulation of the TM quality and market systems, and about 66.7% also agreed on the need for the TM registration system. All regulatory workers who participated in this study at EFDA reported the absence of a registered TM manufacturer's establishment at EFDA (Table 7. 4).

Table 7.4: Responses of the participants regarding the functionality of TM product regulation during the study periods

| Variables                                                       |                                                | Yes |       | No |       |
|-----------------------------------------------------------------|------------------------------------------------|-----|-------|----|-------|
|                                                                 |                                                | n   | %     | n  | %     |
| Registration of TM manufacturing establishment carried-out      |                                                | 50  | 87.7  | 7  | 12.3  |
| Regulation of TM quality conducted                              |                                                | 38  | 66.7  | 19 | 33.3  |
| Regulation of TM market carried-out                             |                                                | 44  | 77.2  | 13 | 22.8  |
| Regulation of TM promotion and advertising carried-out          |                                                | 47  | 82.5  | 10 | 17.5  |
| Regulation of TM clinical trial carried-out                     |                                                | 54  | 94.7  | 3  | 5.3   |
| TM products included in the NEML                                |                                                | 0   | 0.0   | 57 | 100   |
| Inspection of TM manufacturing establishment carried-out        |                                                | 21  | 36.8  | 36 | 63.2  |
| Inspection guideline or SOP for inspectors available            |                                                | 7   | 12.3  | 50 | 87.7  |
| Inspection checklist for TM manufacturer inspectors available   |                                                | 18  | 31.6  | 39 | 68.4  |
| Registered TM product manufacturer in the country               |                                                | 0   | 0.0   | 57 | 100.0 |
| Place and how TM<br>currently sold on the<br>market in Ethiopia | In pharmacies as prescription medicines        | 0   | 0.0   | 57 | 100.0 |
|                                                                 | In pharmacies as non-prescription              | 8   | 14.0  | 49 | 86.0  |
|                                                                 | In other outlets as non-prescription medicines | 6   | 10.5  | 51 | 89.5  |
|                                                                 | In special outlets such as; TM stores          | 10  | 17.5  | 47 | 82.5  |
|                                                                 | In TM service rendering houses as TM           | 57  | 100.0 | 0  | 0.0   |
| No restrictions for selling TM                                  |                                                | 50  | 87.7  | 7  | 12.3  |

NEML: National Essential Medicine List, SOP: Standard Operational Procedures, TM: Traditional medicine.

### 7.3.5. Availability and Functionality of TM Practice and Providers' Regulation Systems by RHRA

Almost all respondents, including RHB personnel, confirmed the existence of a TM regulation system in their respective regions. Similarly, the majority of the respondents agreed on the presence of a system for TM practice registration (88.9%), TM practitioners' registration (98.9%), and TM premise licensing (95.0%). About 76.7% of respondents indicated the absence of inspection guidelines or SOPs for TM practice. Seventy five percent of the RHR officials responded that the measures taken against malpractice related to TM are insufficient (Table 7.5).

Table 7.5: Availability and functionality of regulatory systems for TM practice and provider regulation, by regional officers during the study periods

| Variables                                                  |                                      | Yes |      | No  |      |
|------------------------------------------------------------|--------------------------------------|-----|------|-----|------|
|                                                            |                                      | n   | %    | n   | %    |
| Regulation system for TM products                          |                                      | 179 | 99.4 | 1   | 0.6  |
| Registration system for TM practices                       |                                      | 160 | 88.9 | 20  | 11.1 |
| Registration system for premises of TM practices           |                                      | 171 | 95.0 | 9   | 5.0  |
| PV regulating system that include TM products/preparations |                                      | 2   | 1.1  | 178 | 98.9 |
| TM practices safety regulating system                      |                                      | 142 | 78.9 | 38  | 21.1 |
| TM market regulating and monitoring                        |                                      | 164 | 91.1 | 16  | 8.9  |
| TM promotion and advertising regulating system             |                                      | 144 | 80.0 | 36  | 20.0 |
| TM practices registration currently operated               |                                      | 158 | 87.8 | 22  | 12.2 |
| Inspection plan for TM service rendering establishment     |                                      | 153 | 85.0 | 27  | 15.0 |
| Inspection guidelines or SOP for TM inspectors             |                                      | 42  | 23.3 | 138 | 76.7 |
| Inspection checklists for TM facilities inspectors         |                                      | 166 | 92.2 | 14  | 7.8  |
| Unregistered TMP who currently provide TM service          |                                      | 134 | 74.4 | 46  | 25.6 |
| Registered TMP who provide services unlicensed diseases    |                                      | 128 | 71.1 | 52  | 28.9 |
| TM sells place and market status in selected regions       | In special outlets in like TM stores | 71  | 39.4 | 109 | 60.6 |
|                                                            | In TM establishment as TM product    | 178 | 98.9 | 2   | 1.1  |
|                                                            | No restriction to sell TM            | 156 | 86.7 | 24  | 13.3 |

PV: Pharmacovigilance, SOP: Standard Operational Procedures, TM: Traditional Medicine, TMP: Traditional Medicine practitioners

### 7. 3.6. Overall Performance of the Practical Implementation of The TM Product Regulation

Most of the regulatory professionals at the federal and regional levels (70.2%) responded that the overall performance of the practical implementation of the TM regulation is weak (Figure 5.2).

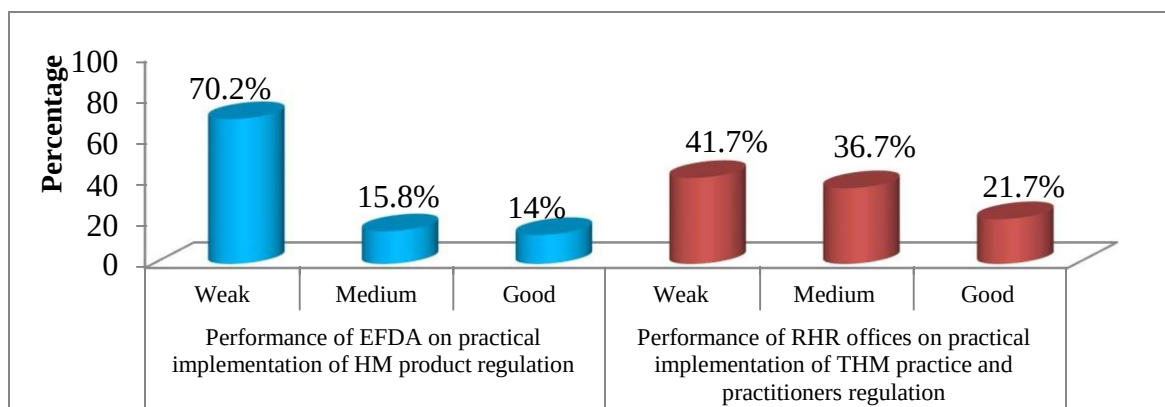


Figure 7.2: The overall performance of the practical implementation of the TM product regulation in Ethiopia during the study period

### **7.3.7. Challenges and Opportunities for TM Regulatory Implementation**

Respondents from federal and regional regulatory authorities indicated that the governing system, human resources, perceptions of TMP towards regulation, and inter-institutional collaboration-related problems were the main identified challenges for implementation TM regulation. The respondents also outlined that those identified challenges are the main bottlenecks for development of TM practices and products in Ethiopia (Table7.6). The presence of a new legislative draft policy, the ongoing establishment of new TM separated offices at the EFDA and MoH, as well as some universities starting to focus on TM research, are some prospect opportunities forwarded by respondents.

Table 7. 6: Current challenges and opportunities to register licenses and regulatory IM systems in Ethiopia during the study periods

| Challenges                                      | Explanation lists                                                                                             | Opportunities Possible solution forwarded                                                        |
|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| System related problems                         | Lack of concerns of overall tradition medicines knowledge and practices of higher officials                   | Draft regulatory policy is on progress                                                           |
|                                                 | Lack of clear separated policy and guidelines                                                                 | Road Map for Research and                                                                        |
|                                                 | Assigning of non-related professionals                                                                        | Development of Traditional Medicine in Ethiopia formulated                                       |
|                                                 | No separate budget allocation for TM practices, regulation and researches                                     | Separate office established under ministry of health                                             |
| Human resource/Regulatory professionals related | No separate education institute and/or no enough curriculum included in health professional study curricula's | Some academic colleges in university and research institutions participating on reach focused TM |
|                                                 | Lack of specialized training                                                                                  | EFDA sponsored regulatory personals is on training                                               |
|                                                 | Deficiency of specialized KAP related to TM                                                                   |                                                                                                  |
|                                                 | Lack of commitment                                                                                            |                                                                                                  |
| TMPs related                                    | Fear of different concerns                                                                                    |                                                                                                  |
|                                                 | Considering TM related activities as secondary works                                                          |                                                                                                  |
|                                                 | Perception of TMPs /practicing TM at secrete place                                                            | Positive perception of TMPs for registration and to be licensed                                  |
|                                                 | Mixing of HM with different colorants to mask the product when applying for license                           | TM contribution in primary health care                                                           |
| Collaboration related                           | Adulteration of HM with MM perceived the same efficacy to enhance activity get more acceptance                |                                                                                                  |
|                                                 | Committing unethical and embractive procedures                                                                |                                                                                                  |
|                                                 | Committing some patient harms after getting license                                                           |                                                                                                  |
|                                                 | Lack of funding organization                                                                                  | EPHI, EFDA and regional health bureaus authorizes                                                |
|                                                 | Lack of collaboration between vertical regulatory bodies from district to federal level                       | starring harmonious collaboration                                                                |
|                                                 | Lack of collaboration between horizontal regional regulatory bodies in licensing and establishing of TMP      |                                                                                                  |
|                                                 | association presence of great conflicts of interests                                                          |                                                                                                  |
|                                                 | Lack of collaboration of regulatory bodies and research institution as well as academic organizations         |                                                                                                  |

TM: Traditional Medicine, TMP: Traditional Medicinal Practitioners, KAP: Knowledge Attitude and Practice, MM: Modern Medicine



Table7. 7- Bivariate and multivariate analysis of factors affecting the performance of regulatory authorities on practical implementation of TM regulatory during study period

| Variables                                           |         | Performance of regulatory offices |                 |               | Crude OR (95% CI) and <i>p</i> -value |              |      |      | Adjusted OR (95%CI) and <i>p</i> -value |             |      |      |
|-----------------------------------------------------|---------|-----------------------------------|-----------------|---------------|---------------------------------------|--------------|------|------|-----------------------------------------|-------------|------|------|
|                                                     |         | Weak<br>n (%)                     | Medium<br>n (%) | Good<br>m (%) | <i>p</i>                              | COR (95% CI) |      |      | <i>p</i>                                | AOR (95%CI) |      |      |
| Awareness or knowledge on TM regulation             | Weak    | 75(61.0)                          | 41(33.3)        | 7(5.7)        | 0.000                                 | 0.18         | 0.10 | 0.34 | 0.009                                   | 0.38        | 0.18 | 0.78 |
|                                                     | Natural | 23(39.7)                          | 20(34.5)        | 15(25.9)      | 0.043                                 | 0.50         | 0.25 | 0.98 | 0.322                                   | 0.68        | 0.32 | 1.46 |
|                                                     | Good    | 17(30.4)                          | 14(25.0)        | 25(44.6)      |                                       | 1            |      |      |                                         | 1           |      |      |
| Government committed and support                    | No      | 98(50.3)                          | 66(33.8)        | 31(15.9)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 75(42.9)                          | 59(33.7)        | 41(23.4)      | 0.002                                 | 2.49         | 1.39 | 4.47 | 0.020                                   | 2.20        | 1.13 | 4.27 |
| Monitoring and evaluation system TM regulation      | No      | 40(64.5)                          | 16(25.8)        | 6(9.7)        |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 60(39.7)                          | 50(33.1)        | 41(27.2)      | 0.000                                 | 3.01         | 1.76 | 5.12 | 0.009                                   | 2.28        | 1.23 | 4.24 |
|                                                     | No      | 55(64.0)                          | 25(29.1)        | 6(7.0)        |                                       | 1            |      |      |                                         | 1           |      |      |
| Lack of adequate and comprehend-save legislative    | No      | 105(50.2)                         | 67(32.1)        | 37(17.7)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 89(53.3)                          | 52(31.1)        | 26(15.6)      | 0.007                                 | 0.48         | 0.29 | 0.82 | 0.126                                   | 0.62        | 0.34 | 1.15 |
| Weak legislative enforcement                        | No      | 26(37.1)                          | 23(32.9)        | 21(30.0)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 108(53.7)                         | 67(33.3)        | 26(12.9)      | 0.000                                 | 0.13         | 0.07 | 0.27 | 0.000                                   | 0.21        | 0.10 | 0.47 |
| Weak cooperation and collaboration on TM regulation | No      | 7(19.4)                           | 8(22.2)         | 21(58.3)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 95(50.5)                          | 65(34.6)        | 28(14.9)      | 0.010                                 | 0.47         | 0.26 | 0.84 | 0.821                                   | 0.92        | 0.45 | 1.88 |
| Lack of adequate basic TM regulatory tools          | No      | 20(40.8)                          | 10(20.4)        | 19(38.8)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 98(51.3)                          | 60(31.4)        | 33(17.3)      | 0.034                                 | 0.52         | 0.29 | 0.95 | 0.067                                   | 0.52        | 0.26 | 1.05 |
|                                                     | No      | 17(37.0)                          | 15(32.6)        | 14(30.4)      |                                       | 1            |      |      |                                         | 1           |      |      |
| Human and financial constraints                     | No      | 40(35.1)                          | 40(35.1)        | 34(29.8)      |                                       | 1            |      |      |                                         | 1           |      |      |
|                                                     | Yes     | 105(52.5)                         | 67(33.5)        | 28(14.0)      | 0.000                                 | 0.21         | 0.11 | 0.42 | 0.001                                   | 0.27        | 0.13 | 0.59 |
|                                                     | No      | 10(27.0)                          | 8(21.6)         | 19(51.4)      |                                       | 1            |      |      |                                         | 1           |      |      |

TM: Traditional Medicine

#### 7.4. Discussion

Traditional medicine in Ethiopia was formally recognized by Proclamation 27/1942. Registration and licensing of TM were also introduced in 1950. During the Derg regime (1970s and 1980s), the promotion and development of TM similarly got official attention with the establishment of the office for the coordination of TM (12,27,28). Despite these facts, TM practice is still overdue. A comparison of the contents of the TM law of Ethiopia compared with five other countries (Kenya, Uganda, Ghana, India, and China), showed that they either integrate TM into national drug/health policies or there are separate exclusive policies designed for TM (3,5,9,29).

All the reviewed and selected countries regulate TM products, practices, and practitioners at the central, state, and lower government health and medicine administration levels. But Ethiopian Proclamation No. 1112/2019 specifies under articles 72/1 and 72/5 that the power and responsibility of TM practices and practitioners' registration and regulation are transferred to RHB, and TM product registration and regulation are held by EFDA, respectively (15,30–32). Ethiopia lacks clear policies on governing bodies' roles, responsibilities, and funding for traditional medicine (TM). While drafts exist for regulation and promotion, implementing them requires collaboration between multiple authorities (8,33,34).

The reviewed African countries, except Ethiopia, define traditional medicine (TM) as indigenous practices and alternative medicine. Indian and Chinese national medicine laws recognize TM as AYUSH and TCM, respectively. Ethiopia has not enacted national regulations for TM practice. The lack of educated TMPs and TM practice development in Ethiopia may be attributed to this discrepancy (35–40). The national TM expert or advisory committee members of all reviewed countries are comprised of MoH, academia, communities, medical practitioners' associations, pharmacy associations, research institutes, and TMP associations (References).

In Ethiopia, as the duty and responsibility of TM regulation is given to regional state, the advisory committee of TM regulation is not established in some regions. Regarding to TM research fund, China and India have both government and public research fund allocations. But, in Ethiopia, the TM research fund is only allocated by the government; whereas, the current status of the research fund in Kenya, Uganda, and Ghana is not

clear(8,16,28,31,41,42). Ethiopia lacks regulation policies for veterinary, crude, and imported TM products. GMP requirements for TM manufacturing in Kenya, Uganda, Ghana, India, and China are in the implementation stage. However, Ethiopia has drafted separated TM regulation during the study period (43–48).

The reviewed countries have unique safety requirements for TM products; Ethiopia relies on ethnomedicinal information and regional state regulation (36,49–52). Traditional medicine education is offered at the university level in Ghana, India, and China, with Ghana offering a four-year Bachelor of Science in TM, while India and China offer bachelor's, master's, and PhD degrees (2,3,29). With the exception of a few short training programs linked to indigenous TM and courses included in bachelor's and MSc-level pharmacy curricula, Ethiopia currently lacks a structured academic institute that primarily conducts TM-related courses. Health insurance does not cover TM services in Ethiopia, Kenya, and Uganda. In India, Ghana, and China, TM services are reimbursed by public and private insurance (8,36). In all selected countries, the associations of TM practitioners are considered NGOs. In Ethiopia, the TMP's associations have poor collaboration across the country (35,40,50,51).

Ethiopia's health proclamation lacks clear definitions for minerals, animal products, dietary supplements, functional foods, and natural supplements compared to other countries (8,22,37,47). Ethiopia lacks provisions for controlling veterinary medicines, with VDAFACA regulating production and practices resulting in poor performance compared to other countries (47,53,54). Ethiopia's FDA regulates category 3(preparet TM crude product) and 4(active isolated pure compounds) TM products, while RHR controls category 1 and 2 TM items. Around 81% of EFDA regulatory staff report there is some trial to distinctly regulatory structure for TM products. However, there is a lack of a pharmacovigilance reporting system (8,37,52,55,56).

This is obvious because of the absence of a legal framework and policy guidelines. The majority of EFDA regulatory employees in Ethiopia replied the lack of prescriptions and any claims-based TM regulation category, the lack of SOP standards, MA checklists, and TM

product inspection. This demonstrated that the practical application of TM product regulation in Ethiopia is at its infancy stage unlike to other reviewed African countries. However, the ongoing efforts in the preparation of regulatory guidelines by the EFDA to support the implementation of TM product regulation are of good promise (28,42,47,49,53,57,58).

Table 7.7 shows challenges for TM regulation implementation, including the governing system, human resource concerns, perceptions of TMP, and inter-institutional collaboration. Most respondents recognized these issues as barriers for regulation, training, research, and development, as well as the commercialization and deployment of TM in Ethiopia. Lack of separated offices, lack of budget allocation, poor policy guidelines, and the absence of TM education institutes contribute to government system-related problems in TM regulation, research, and development. Previous studies in African countries and Malaysia also highlight the same challenges. Inadequate TM related training, lack of commitment by some regulatory officers at all levels, fear of different concerns to approve TMP licensing (for example, fear of illegal actions or harming clients by TMP), and viewing TM-related activities as secondary works are some human resource and regulatory professional-related issues that have mostly been addressed by regulatory managers. Some of these issues were also noted in some previous studies (37,41,59,60).

The respondent addressed TMP-related problems related to the regulation of TM, adulteration of TM with conventional medicines, unethical procedures after obtaining a license, considering registration and licensing as punishment, and governmental tax collection, highlighting the potential loss of efficacy if the TM secret is revealed. Some of these views were similarly found in previous studies. Respondents also identified collaboration-related issues such as a lack of national and international financing partners, conflicts of interest between regional and federal TMP associations, and weak collaboration between regulatory agencies and research and academic institutions. These finding is against some previous similar studies (3,37,55,61). The deviation may be due to advanced TM practice technology in selected and compared countries and governing structure of the regulatory authorities. Ethiopia's potential opportunities for TM regulation implementation include a draft regulatory policy, an approved research roadmap, a separate TM office under the MoH, universities

involvement to TM related researches, positive perceptions of some TMP for registration and licensing, and harmonious collaboration between federal and regional health bureau authorities (16,28,31,53,62). All the findings of the current KI interview complemented the archival review findings.

In bivariate ordinal logistic regression analysis, factors that indicated a significant association ( $p < 0.05$  at 95% CI) with the performance level of regulatory offices were chosen for multivariate ordinal logistic regression analysis. As a result, regulatory officers' knowledge and commitment, as well as weak legislative enforcement and resource constraints, were identified as significant associated factors for the implementation of TM regulation in Ethiopia (Table 7.7), which is consistent with a similar study conducted in some other countries as indicated in the WHO survey report (5,35,59,61,63).

Keeping other parameters constant, the performance of the regulatory office for the implementation of TM regulation was 2.20 times more effective with good support and commitment from the government (AOR = 2.20, 95% CI: 1.13–4.27),  $p = 0.020$ ). The presence of an effective monitoring and evaluation system on TM regulatory activities was associated 2.28 times with the achievement of good performance in TM regulatory implementation as compared with the absence of an effective monitoring and evaluation system (AOR = 2.28, 95% CI: 1.23–4.24,  $p = 0.009$ ). The presence of resource (human and financial) constraints affects 0.27 (73%) times the probability of having good performance in implementing TM regulation than the resource constraint solved (AOR 0.27, 95% CI: (0.13–0.59));  $p = 0.001$ ). This implies that when a unit of human resource and financial problems is solved for regulatory offices, we can expect 3.71 times higher TM regulatory implementation while all the other variables in the model are held constant.

## **7.5. Conclusion and Recommendations**

Ethiopia formulated a health policy that included TM in 1993, which was reviewed and changed in EFDA Proclamation No. 1112/2019 to control both MMs and TM. Due to the lack of an exclusive regulatory framework for TM in Ethiopia, TM products and practices are controlled holistically with conventional medicine under one organizational structure. Proclamation No. 1112/2019 is insufficient and not comprehensive on the type

of TM product regulation. Currently, EFDA has drafted an exclusive TM policy and proclamation. Traditional Medicine is sold in public areas by unregistered practitioners, with most TMPs engaging in TM practice without registration or licenses. The implementation of TM products, practices, and providers is in its infancy, with significant factors affecting performance. The governing system, human resources, perceptions of TMPs, and inter-institutional collaboration challenges TM regulationimplementation.

Monitoring and evaluating the safety, efficacy, and quality of TM sold in the market is crucial. Basic training for TMP and continuous on-the-job regulatory staff training is necessary. A formal TM curriculum and training school should be established in Ethiopia. Issues related to TM integration with mM, and patient referral systems should be addressed. A comprehensive national TM standard of practice (SOP) and pharmacopeia should be developed, and a national TM association should be established. Stakeholders should work together to improve TM quality, safety, and effectiveness of TM in Ethiopia.

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## **CHAPTER VIII: GENERAL DISCUSSION**

### 8.1. Overall Discussion

Plant-based TM has been utilized for generations for the treatment of different perceived infections. However, ethnomedicinal information is predominantly transmitted orally, posing a risk of potential loss (1–4). Most previous researches in Ethiopia focused on survey types. The information was fragmented, with insufficient scientific data (5–8). The goal of this PhD study was to generate knowledge about ethnomedicinal plants used to treat perceived infectious diseases, determine the bioactivity, phytochemical components, of selected medicinal plants, and to investigate the regulatory status of TM in Ethiopia. Thus, it integrated various basic TM research aspects into a single table to fill the gaps boldly evidenced related to TM research problems in Ethiopia.

The study identified various plant families and species, socioeconomic characteristics of TMPs, and indigenous beliefs regarding disease causation. The study found that most TMPs were elderly, living in rural areas with low incomes, while the younger generations showed little interest in ethnomedicinal practice. The study identified indigenous designations given by TMPs and the surrounding communities in their local languages for various infectious diseases. These include male disease (*Dhukuba dhiiraa*), evil eye (*Ija namaa=buda*), Michii (sudden onset of respiratory tract illness), severe itching (*Ooqxoo*), severe cough (*Qufaa qorsaa*), shivering (*rommisisaa*), night bird disease or bat urine disease (*Fincooftu halkanii*), rainbow disease (*Sabbata Waaqayyoo*), and necrotizing wounds caused by insect bites (*tibijiitizing*). Except for language and certain designation discrepancies, this study is consistent with the traditional beliefs and sickness-curing techniques of other studies conducted in Ethiopia. Some indigenous designations and perceptions of diseases are related to modern medical theories, but some of the traditional beliefs about infection need correction through training (8,9). The study also revealed that the communities prefer ethnomedicinal medicine due to its affordability, availability, and perceived effectiveness. The majorities of TMPs have inherited ~~the~~ traditional knowledge from family members and have passed it down to their children only. Most of the TMPs collect medicinal plants from the wild, but difficulties arise from deforestation and agricultural expansion. The findings are similar to some previous studies (16–19). The most common plant families used for human infection treatment include

Asteraceae, Fabaceae, Euphorbiaceae, and Lamiaceae. *Vernonia amygdalina* Delile

(Eebicha), *Croton macrostachyus* Hochst. Ex Delile (Bakkaniisa), and *Ocimum gratissium* L (Damakase) were the most commonly reported plant species for the treatment of different infections in our study. These plants are used to treat various diseases, including intestinal parasites, skin diseases, respiratory tract infections, sexually transmitted infections, gastrointestinal infections, rabies, and neglected tropical diseases, and they were in line with previous similar studies (10–13). Leaves and, roots, were the most used plant parts used and oral as well as dermal routes being the most common used routes of administration. Most medicinal plant oral preparations are combined with water, coffee, honey, and milk, while dermal treatments are primarily combined with butter and honey. In contrast with other countries with advanced technologies (14,15).

Most plants selected and tested for secondary metabolites exhibited different phytochemical classes of compounds, including flavonoids, alkaloids, phenols, glycosides, and steroids. Regarding the antimicrobial test, the crude extracts of *T. rhynchocarpum* root demonstrated the greatest antimicrobial efficacy, with the lowest *in-vitro* inhibitory concentration ( $<10 \mu\text{g/ml}$ ). Additionally, the chloroform fraction of *J. schimperiana* showed antibacterial activity. The observed biological activity profile could be due to the individual class of compounds present in each plant or the synergistic effect of each class of compounds (20–23).

Synergistic interactions between selected plant extracts and conventional antibiotics can be effective in combating antibacterial resistance. Combination therapy offers advantages over monotherapy by increasing efficiency, reducing side effects, maintaining stability, improving bioavailability, and enabling the use of lower doses compared to synthetic alternatives. It can offer an economic advantage over traditional drug discovery as it restores an existing drug to reduced resistance, allowing for faster clinical usage and lower development costs (24,25). Over the past decade, studies have shown a significant increase in the synergistic interaction between plant extracts and antibiotics. Combinations of antibiotics and plant extracts have shown significant antimicrobial activity against multi-resistant strains (26–29). In this PhD



study, the findings show that combining plant extracts enhances the efficacy of tested antibiotics. The study evaluates the MICs of three antibiotics (Tetracycline, Amoxicillin, and Cloxacillin) in conjunction with three plant extracts chloroform fraction of *C. abyssinicum* Hochst. ex A. Rich, chloroform fraction of *C. hirsuta* Guill. and Perr, and *T. rhynchocharpum* Quart.-Dill. & A. Rich crude extract) to assess their combined effects against *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 43300). These plant extracts and antibiotic combinations showed a promising fractional inhibitory concentration (FIC) index ( $< 0.5$ ). Tetracycline and *C. abyssinicum* Hochst. ex A. Rich showed additive effects. Combining Cloxacillin with these extracts increased its efficacy by 8 and 16, folds against *P. aeruginosa* and *S. aureus*, respectively, and this is consistent with prior research (26,30,31).

Plant products are valuable sources of therapeutic agents in most modern drugs, and recent scientific developments and technological advances suggest that natural products will be important sources of new drugs in the future. The development of novel, efficient, and cost-effective medications is essential for combating microbial infections, particularly in less developed countries (32,33). In Ethiopia, even if plant products have been crucial in treating human and animal illnesses, only a small portion of natural compounds have been isolated. In this PhD study, we isolated three compounds from the root bulb of the dichloromethane fraction of *C. abyssinicum*. Compound 1 was isolated as a whitish solid with an  $R_f$  value of 0.7 in n-hexane:ethyl acetate (7:3). Its ESI-MS data analysis suggested the molecular formula for  $C_{16}H_9NO_3$ . The data from the NMR spectra supports the structure of compound 1, which is identified as an alkaloid with a pyrrolophenanthridone nucleus (Lycorine type) named hippadine, as evidenced by the previous study (34,35). Compound 2 was isolated as a solid powder with an  $R_f$  value of 0.52 in the n-hexane/ethyl acetate (1:4) solvent system. Based on the NMR spectra, compound 2 is characterized by myrianthic acid; it was previously reported from the leaves of *Campsis grandiflora* but is new from *C. abyssinicum* (36). Compound 3 was isolated as a whitish crystal weighing 6 mg in a solvent system of 20% n-hexane and 80% ethyl acetate. The  $^1H$  NMR spectrum data confirmed that the compound's structure is 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone). This compound was previously reported from *Vismia baccifera* but it is new to this plant species (37).

The absence of a robust regulatory framework can have detrimental effects on both human and animal health. Governments must develop genuine policies and implement them

effectively to safeguard the well-being of their citizens. The legal basis and practical implementation of TM regulation in Ethiopia require a thorough assessment (9,38). Our study identified that TM regulation in Ethiopia is still underdeveloped. Despite being recognized by Proclamation 27/1942 and registration and licensing introduced in 1950, Ethiopia has not enacted national regulations for TM practice. The country lacks clear regulations for veterinary, crude, and imported TM products, and the implementation of these requires collaboration between multiple authorities. Traditional medicine Practitioners associations have poor collaboration across the country. Ethiopia's regulatory system faces challenges such as lack of separated offices, poor budget allocation, poor policy guidelines, and the absence of TM education institutes. Potential opportunities for TM regulation implementation include a draft regulatory policy, an approved research roadmap, a separate TM office under the MoH, university involvement in TM-related research, positive perceptions of some TMP for registration and licensing

## **8.2. General Conclusion**

This PhD thesis generated knowledge related to selected medicinal plants used for the treatment of infectious diseases, their antimicrobial activity status, and the components of secondary metabolites present in selected medicinal plants.

In **Chapter I**, we presented global infectious disease patterns, and antibiotic discovery trends from a global to a local level. And also the contribution of ethnomedicine for discovering of active antimicrobial products was briefed. In **Chapter II**, research questions and objective of the study stated. In **Chapter III** general methodology of the study was explained.

**Chapter IV** generated knowledge related to ethnomedicinal plants and associated indigenous knowledge used by TMPs to diagnose, prevent, and treat perceived infectious diseases. A study identified 164 TMPs and indigenous designations in the local language for various infectious diseases, including male disease, evil eye, sudden onset of respiratory tract illness, severe itching, cough, shivering, night bird disease, rainbow disease, and so on. Seventy ethnomedicinal plant species from 40 plant families were identified. The most commonly reported plant species were *V. amygdalina* Delile, *C. macrostachyus* Hochst. ex Delile, and *O. gratissimum* L. Wild pig meat and Ethiopian Hedgehog meat were also used for human infection treatment. Leaves and roots were the most commonly used plant parts; oral and dermal were the most commonly used routes of administration. The study reveals that TM is

preferred by communities over modern medicine due to consumer trust, affordability, and ease of access. Intestinal parasites, gastrointestinal infections, respiratory infections, leishmaniasis, and rabies were among the most common infections for which these plants were used. Traditional medicinal practitioners primarily learn from family members and share their knowledge verbally with the selected family members. Younger generations are reluctant to practice TM. Only 28% of TMPs are willing to disclose their practices, citing fear of ineffective treatment and their main source of income. The study found that 83% of TMPs use herbal preparations for the treatment of perceived infections. However, 67% of them face accessibility issues due to deforestation and agricultural expansion. Only 29% of people conserve plants in their gardens. The study also assessed TMPs' perceptions of training, regulation, and integration into the health system. Eighty-two percent of respondents agreed on the need for training, 76% agreed on the integration of TM with MM, and 73% expressed support for ethnomedicinal regulation. The literature search part showed that the majority of the previous studies were inventory types. The study provides scientifically sound information that natural products from Ethiopian medicinal plants are capable of treating various infectious diseases. However, these plants have not been thoroughly explored and documented, and information on the efficacy, safety, and quality of these plants is held in secrecy.

In **Chapter V**, the secondary metabolites (phytochemical components) and the antimicrobial activity of selected medicinal plants were identified. The finding, alkaloids, phenols, glycosides, and steroids are more abundant. The study also found that all fractions of *T. rhynchocarpum* root extract showed the promising activity. The chloroform fraction of *J. schimperiana* demonstrated good antibacterial activity against *S. aureus* and *E. coli*, with MIC values below 10 µg/ml. Extracts from *S. nigrum*, *D. steudneri*, *T. dregae*, and *M. foetida* showed insignificant activity. The observed biological activity profile could be due to either the individual class of compounds present in each plant or the synergistic effect of each class of compounds. The study combines three antibiotics (Tetracycline, Amoxicillin, and Cloxacillin) with tree plant extracts to determine their effects against *P. eruginosa* and *S. aureus*. The plant extract and antibiotic showed a promising fractional inhibitory concentration (FIC) index. All three plant extracts showed synergistic effects with tested antibiotics with an FIC value < 0.5. The amoxicillin activity was enhanced by 32 and 64 folds

against *P. eruginosa* when combined with *C. hirsuta* Guill, Perr, and *T. rhynchocarpum* Quart.-Dill. & A. Richtimes, respectively. Hence the interaction between tested antibiotics and plant extracts was beneficial in boosting the efficiency of antimicrobial agents. This synergy between antibiotics and plant extracts may help resolve the effectiveness and toxicity issues caused by excessive antibiotic dosages.

**Chapter VI** focused on the isolation of an active potential anti-microbial compound from the root bulb of *C. abyssinicum*. The plant material was extracted fractionated and pure compounds were isolated using column chromatography. Structural elucidations were performed using NMR spectroscopy. The chloroform fraction displayed the highest level of activity and yielded three compounds. Compound (1) is isolated as an whitish solid as an alkaloid with a pyrrolophenanthridone nucleus (Lycorine type) named Hippadine (C<sub>16</sub>H<sub>9</sub>NO<sub>3</sub>). The second compound (2), a 9mg brown solid powder, was isolated as 2, 3, 19, 23-tetrahydroxyurs-12-en-28-oic acid (myrianthic acid). The third compound (3) isolated a whitish powder as 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone). The *in vitro* MIC of antibacterial and anti-Leishmanial activity of different fractions and pure compounds of the root bulb of *C. abyssinicum* showed promising activity against tested microorganisms. The chloroform portion had the highest activity, with a MIC of 1.9 µg/ml against *S. enterics* and 3.9 µg/ml against *S. styph* and *Sh. flexneri*. All fractions of the plant extract and isolated compounds exhibited moderate antimicrobial activity.

In **chapter VII**, the TM legal framework and its practical implementation status in Ethiopia were assessed. Accordingly, TM is regulated along with allopathic medicine under Proclamation No. 1112/2019. It is. According to this study TM medicine regulatory framework in Ethiopia is insufficient and has a lot of gabs including: There is no officially registered TM product; Traditional medicine training is not yet authorized; Health insurance coverage for TM healthcare is not available; Ethiopia's TM policy lacks a code of ethics, a national expert committee, and information on TM integration into health service delivery.

The majority of EFDA regulatory personnel stated that pharmacovigilance system is only available for conventional pharmaceuticals. Regulation of TM and both federal and regional levels is weak. The main challenges for implementing TM regulation in Ethiopia include the governing system, human resources, TMP perceptions, and inter-institutional collaboration issues. Opportunities include a new legislative draft policy, the presence of TM research and

development road map, and opened new separated TM offices. The study found regulatory officers' knowledge and commitment, weak legislative enforcement, and resource constraints significantly impact the implementation of TM regulation in Ethiopia. Good government support and commitment were found to be more significant for the implementation of TM support. An effective monitoring and evaluation system was also associated with better performance.

In **Chapter VIII**, we summarized the study, stated the implications of the findings, highlighted the limitations of the study, and forwarded recommendations and broader international context, relevance, and future research prospects. Implication for the findings

This PhD thesis has significant impacts on TM practice, research, and policy issues in Ethiopia. Traditional medicine is threatened by factors like the disappearance of old TMPs, agricultural expansion, population dynamics, urbanization, environmental degradation, and overuse of medicinal plants. Despite the abundance of medicinal plants and the fact that the majority of Ethiopians rely on TM, only a tiny portion of it is studied, and it has not been scientifically developed and incorporated into primary health care usage. A full- information database of documented medicinal plant species is also lacking. Antimicrobial activity test findings, phytochemical screening, and the isolation of active pure components are not satisfactory. The status of the TM regulation's practical implementation is unknown in Ethiopia. Therefore, this study generated knowledge of ethnomedicinal information, bioactivity studies, and secondary metabolites present in selected medicinal plants used for the threat of different perceived infectious diseases in Ethiopia. A better understanding of the natural resources that are used as raw materials in the production of microbiological drugs was obtained from study area. It also identified the scope and goals of TM regulation in Ethiopia.

The information about TM and associated indigenous knowledge was comprehensively collected, documented, and evidenced to add to the database of TM in the country. The active pure active compound was isolated, and its molecular structure was elucidated and made ready for the next study of drug development. The legal framework and its practical implementation status for the regulation of TM in Ethiopia were also assessed. This study is a new approach that brings different aspects of TM research information into a single table (the gathering of full information about ethnomedicinal knowledge, bioactivity tests, molecular

characterization, and regulatory status of TM, as well as plant extract and conventional antibiotic combination synergistic effect analysis). This makes our study innovative and one step forward from previous TM studies. The finding provided scientific evidence for the use of selected traditionally used medicinal plants. It laid the foundation for the integration of TM with modern medicine in Ethiopia. It also lays the groundwork for the identification of low-cost, active antibacterial lead compounds, which will aid in the beginning of the integration of TM practice with modern medicine (MM). It provided information for research institutes, policymakers, and the pharmaceutical industry about TMs for the treatment of different human infections. It is the base line for further research to conduct modern drug and plant extract synergy for the re-using and /or re-purposing of the activity of conventional antibiotics and to reach clinical usage much more rapidly and at a much lower development cost. To address boosting of antibiotic activities, new approaches are needed, such as using plant extracts alone or in combination with conventional antibiotics. Our study prioritizes antimicrobial natural product combination drugs due to their economic advantage.

The study also reveals that TMPs in Ethiopia have valuable skills. But they are elderly and live in low-income areas with unexplored, in-depth knowledge of diagnosing and treating different diseases. The study also highlights new claims of plants in Ethiopian forest biodiversity that are most effective in treating different infections. Since traditional knowledge of medicinal plants in Ethiopia plays a crucial role in primary care for treating human ailments, the documentation and conservation of these resources should be a priority area. The generated knowledge can also be used to teach undergraduate and postgraduate students from different departments and for TMP training. Finally, this study also provided evidence about the practical implementation of the existing TM regulatory system that promotes safe, effective, and quality TM knowledge, practices, and products. Ethiopia's TM regulatory framework is inadequate for ensuring the quality, safety, effectiveness, and reasonable use of TM. The present medical policy encompasses both conventional and TM; however, implementation is in its early stages. Unregistered practitioners sell TM in public places without registration or licensing. A legislative framework, consistent organizational structures, and practical execution in regional states are required to assure public safety. There are several unsolved questions about TM regulation that require much further effort at the federal and regional government levels. Then the finding can be important for revising and developing national TM policy.

## 8.2. Limitations of the Study

*In vitro*, only antimicrobial testing methodologies may accurately not represent the biological efficacy of the tested extracts. Extraction by maceration and conventional column chromatography isolation methods is an old method and can affect the yield and components of active extract outputs. The self-administered questionnaires filled out by regulatory officers and management may be biased and conceal certain important facts.

## 8. 4. Recommendations

Since this PhD thesis mainly focused on medicinal plants traditionally used for treating perceived infectious diseases, a comprehensive national-wide ethnomedicinal study is needed to document disappearing medicinal plants and related knowledge. *In vitro* antimicrobial studies of selected medicinal plants should be conducted on other untested bacterial strains. Based on traditional claim further *in vivo* antimicrobial, anti-viral, anti-cancer, anti-inflammatory, and anti-oxidant, anti- diabetic, and other bioassays should be conducted, especially using *C. abyssinicum* Hochst. Ex A. Rich. Structural modification of isolated compounds is needed to enhance their activity.

A national ethnomedicinal plants and associated knowledge database should be established to make related data easily available for further research. Research institutions, the Ministry of Health, and Universities must finance and coordinate the establishment of *in situ* and *ex situ* conservation and propagation centers to conserve endangered medicine plans in Ethiopia. A new initiative should be started by the MoH and concerned bodies for the integration of TM with the primary health care of modern medicine. General school syllabuses should incorporate indigenous knowledge and TM practices for the teaching of an emerging new generation of Ethiopian citizens. The Minister of Health and the MoE should collaborate to develop legislation and programs for formal TM education and training. Traditional medicine-related training must be widely given to concerned departments by expanding the available curricula. On-the-job training related to TM must be given to voluntary health providers. Independent TM undergraduate and post-graduate TM training institutions must be opened at the national and/or regional level. Short- and long-term training curricula must be developed, and continuous training should be provided for TMPs. Traditional medicine research and development centers must be established in selected universities, and TMP must be encouraged, supported, and regulated to open legal TM service centers. Extensive

pharmacological, toxicological, and phytochemical studies should be the potential focus of herbal medicine, which could be the baseline for the integration of herbal medicine with modern medicine in Ethiopia.

The Food and Drug Authority of Ethiopia should provide a suitable and effective legal framework for regulating, promoting, and developing TM practices and products. Establishing an exclusive organizational structure for TM product promotion and regulation is needed. Developing and ratifying TM-related update policies, proclamations, regulatory tools, patent law, and other legal frameworks by cooperating with others stakeholders is are essential. For researchers and scholars, further studies should be done including officials not included in this study, such as manufacturers, unregistered TM practitioners, end-users of TM, lower administrative levels of regulatory officials, and so on, to explore new insights. Researchers and scholars should be supported in the preparation of TM-related guidelines, regulatory tools, pharmacopeia, monographs, standards, checklists, and other TM-related documents and activities. All concerned stakeholders directly and/or indirectly involved in TM products, practices, and providers in Ethiopia should work together to realize the development, promotion, and regulation of TM and improve the quality, safety, and effectiveness of TM availability on the markets of Ethiopia that enhance public health and the economic growth of the country.

#### **8.5. Broader International Context, Relevance and Future Research Prospective**

The Alma-Ata Declaration (1978) emerged as a major milestone of the twentieth century in the field of public health, and it identified primary health care as the key to the attainment of the goal of health for all. Primary health care is essential health care based on practical, scientifically sound, and socially acceptable methods and technology made universally accessible to individuals and families in the community through their full participation and at a cost that the community and country can afford to maintain at every stage of their development in the spirit of self-reliance and self-determination (39–41). It forms an integral part both of the country's health system, of which it is the central function and main focus, and of the overall social and economic development of the community. It is the first level of contact of individuals, families, and communities with the national health system, bringing health care as close as possible to where people live and work and constituting the first elements of a continuing health care process. The current SDG 3 also aspires to ensure health



and well-being for all, including a bold commitment to end the epidemic of communicable diseases by 2030. It also aims to achieve universal health coverage and provide access to safe and effective medicines and vaccines for all (39,42,43).

Traditional medicine is often seen as more accessible, more affordable, and more acceptable to local populations and can therefore be a tool to help achieve universal health coverage. Towards universal health coverage: advancing the development and use of TM is important. Progress is evident, especially in sub-Saharan African countries, in TM research and development. Collaboration between TMP and conventional health practitioners, quality assurance, regulation, registration, and TM integration into national health systems are mandatory. Despite the fact that many nations worked hard following the declaration to build an integrated healthcare strategy to provide safe, high-quality, effective, and cheap TMs services and products, the outcome is unattractive. The demand for TM has recently increased because of public health concerns and economic values. International concern and collaboration should be expanded for TM education, research, and standardization of TM products to facilitate their access to them for long-term development.

This PhD study highlights the scientific evaluation of ethnomedicinal plants and their related indigenous uses. It highlighted the potential medicinal plant species to combat infectious disease. It identified active secondary metabolites and pure active chemical components present in selected plant extracts and finally explored challenges and prospects for TM regulation in Ethiopia. The outlook for the future resulting from this PhD work can be exemplified by the following prospects: in collaboration with stakeholders

- 1) An herbal medicine garden has been established in the Mettu University main campus.
  - ✓ Medicinal plants identified by the ethnomedicinal survey, including endangered ones, will be collected and planted.
  - ✓ Further studies will be conducted on planted species.
  - ✓ Cultivation and propagation will be focused on adapted and conserved medicinal plants.
  - ✓ Ex-situ conservation sites will be selected and established in surrounding community institutions (primary and secondary schools, agricultural research centers, and green parks).
- 2) A TM research and development center will be established at Mettu University to conduct further advanced TM research

- ✓ An advanced pharmaceutical chemistry lab is started in the Mattu University Pharmacy Department.
- ✓ Students and junior staff will be trained on TM research from different universities and institutes..
- 3) A postgraduate program in herbal medicine and drug development will be launched.
- ✓ Curriculum development is accomplished
- ✓ Traditional medicine-related multidisciplinary postgraduate programs will be launched in the near future.

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12. Shafiq A, Muhammad Z, Shehla S, Mushtaq A, Zabta Khan S, Shazia S, Et Al. Ethno-Medicinal Plants And Traditional Knowledge Linked to Primary Health Care Among The Indigenous Communities Living In Western Hilly Slopes of Dera Ghazi Khan, Pakistan. 2020;(March).
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23. Asfere Y, Kebede A, Zinabu D. In-vitro Antimicrobial Activities and Phytochemical Screening of Selected Plant Extracts against Some Medically and Agriculturally Important Pathogens. *European Journal of Medicinal Plants*. 2020;31(10):167–89.
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## Curriculum Vitae of the Author

### Curriculum Vitae

#### Personal information

|                              |                                              |                                                                                     |
|------------------------------|----------------------------------------------|-------------------------------------------------------------------------------------|
| Full name:                   | Sileshi Dubale Dinssa                        |  |
| Sex                          | Male                                         |                                                                                     |
| Professional title/Position: | Pharmacy                                     |                                                                                     |
| Highest Degree:              | MSC                                          |                                                                                     |
| College                      | Health science                               |                                                                                     |
| Affiliation:                 | Mettu University, Mettu Ethiopia             |                                                                                     |
| Nationality:                 | Ethiopian                                    |                                                                                     |
| Marital status               | Married                                      |                                                                                     |
| Number of children           | Two                                          |                                                                                     |
| Language                     | Excellent in Afaan Oromoo, Amharic & English |                                                                                     |

#### Educational status

| Year       | Education                   | University /institution    |
|------------|-----------------------------|----------------------------|
| 2013- 2015 | M.Sc. in Clinical pharmacy  | Jimma University           |
| 2004 -2010 | Bachelor degree in pharmacy | Jimma University           |
| 2001- 2002 | Diploma in clinical nursing | Addis Ababa University     |
| 1997– 2000 | Grade 9-12                  | Mettu secondary school     |
| 1995-1996  | Grade 7-8                   | Mettu Abune Petiros school |
| 1989-1994  | Grade 1-6                   | Alebuya primary school     |

#### III) Working Experience

9/5/2003 -4/6/2007(4 years) Jimma University Specialized Hospital, as clinician

5/6/2007-13/11/2011(4yers) Technical Assistant at Jimma University Nursing school

14/11/2011-1/4/2012 (2years) Jimma University Jimma University Pharmacy school Assistant lecturer

13/11/2012-1/11/2013(1 Year) Health scince faculty cordnater, assistant lecturer at Mettu University health science faculty

20/3/2015-1/1/2018 (3years) Lecturer, Researcher, Adviser and Community Service and Consultancy Directorate

Director, Mettu University

Current status: Assistant professor of pharmacy at Mettu University

Positions Held and Extracurricular activities and Ngo related jobs

Community Service and Consultancy Directorate Director, Mettu University

Voluntary board member of Ethiopian Red cross Ilubabor Zone branch

Per time training of ART, PMCT and Chile care at Ethiopian Evangelical Church Mekane Yesus Development and Social Services Commission (Eecmy-Dassc) Jimma branch

Per time child support and protection training at Ethiopian Evangelical Church Mekane Yesus Development and Social Services Commission (Eecmy-Dassc) at Ilubabor bethel synodos branch



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### Selected Trainings

Managing and designing project online trading Provider cornerstone on Demand foundation; coalition date Feb 2 and 9.,2023 (online via DisasterReady.org)

“Humanitarian Basics” online training, Provider cornerstone on Demand foundation; coalition date January 30,2023 (online via DisasterReady.org)

“Grant Writing training”, organized by Texas Tech University and Jimma University, Jimma Ethiopia, August 12th -15th /2019

“ Research stay and training on Natural product for anti-bacterial and anti-parasite”, organized and funded by DAAD/YaBiNaPA, Yaoundé Cameroon, from June 1<sup>st</sup> – November 30<sup>th</sup> 2018

“Clinical teaching skill training” organized by Jhpiego, Ministry of Health and Ministry of education, Jimma Ethiopia, February 1-3/2010

“Effective teaching skills for Medical faculty instructors”, organized by Jhpiego, Ministry of Health and Ministry of education, Jimma Ethiopia, February 25-March 01/2009

“Student performance Assessment Training”, organized by Jhpiego, Ministry of Health and Ministry of education, Jimma Ethiopia July10-12/2009

“Online coarse creation and management” Jimma University, Jimma Ethiopia, Nov. 07-11/2011

“IT essentials for instructors” for three months Jimma University and CISCO academy Jimma Ethiopia awarded on Jun 8 2013

“HIV/AIDS, infection prevention and record keeping”, Jimma University and Nottingham city Hospital; 26 October -7<sup>th</sup> November /2003 Jimma Ethiopia

VCT post basic course organized by Ministry of Health and CDC, Addis Abeba Ethiopia, from November 8-19/2004

HIV/AIDS Knowledge update on PMCTCT and ART organized by Jhpiego, Ministry of Health and Ministry of education, Jimma Ethiopia, September 3-7/ 2008“

“Use of Ultrasonography to study abnormal pathology” organized by IUC-JU project, July14-18/2008

Basic training on infection prevention and patient safety for higher education teaching staffs organized by AIDSTAR-ONE Ethiopia Jimma Ethiopia, July 6-10/2011

### Research Experience

Participated in different staff researches,

Advised different health science under graduate and post graduate students

More than ten research publication on peer reviewed and reputable journals

### References

Prof.: Sultan Suleman email [sultan.sulemanl@gmail.com](mailto:sultan.sulemanl@gmail.com)

DR Endegen Abebe email [endgena@meu.edu.et](mailto:endgena@meu.edu.et)

Dr Negera Abdisa Tele: email [negeraabdisa@gmail.com](mailto:negeraabdisa@gmail.com)

### Current address

SILESHI DUBALE DINSSA Tel.+251 917 80 60 80

Email [sileshi.dubale@gmail.com](mailto:sileshi.dubale@gmail.com) /[sileshi.dubale@meu.edu.et](mailto:sileshi.dubale@meu.edu.et) Mettu Ethiopia

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**Signature and Certification**

I attest that the information in my personal profile is accurate, complete, and truthful. I also attest to the authenticity and accuracy of any papers I may be asked to submit in support of my application. I am aware that making a false or misleading statement or omitting important facts could result in the withdrawal of an appointment offer or the termination of my employment. As a result, this position matched my level of enthusiasm and dedication, experience, and academic qualifications to serve the organization and the community honestly in accordance with its vision, aims, and objectives. So, I respectfully ask that you take my candidacy for this position into consideration.

Sincerely,

Mr. Sileshi Dubale

Date -----

## Lists of Publications

1. Usure RE, Kebebe D, Mekasha YT, Hasen G, ChuraWaritu N, **Dubale S** and Suleman S (2024), Traditional herbal medicine regulatory implementation in Ethiopia: a qualitative study. *Front. Pharmacol.* 15:1392330. doi: 10.3389/fphar.2024.1392330
2. **Dubale, S.**, Abdissa, N., Kebebe, D., Debella, A., Zeynudin, A., & Suleman, S. (2023). Ethnomedicinal plants and associated indigenous knowledge for the treatment of different infectious diseases in Ethiopia. *Journal of Herbal Medicine*, 40(May), 100669. <https://doi.org/10.1016/j.hermed.2023.100669>
3. **Dubale S**, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*.
4. Jifar WW, Geneti GB, **Dinssa SD**. The Impact of COVID-19 on Pharmaceutical Shortages and Supply Disruptions for Non-Communicable Diseases Among Public Hospitals of South West, Oromia, Ethiopia. *J Multidiscip Healthc.* 2022 Aug 31;15:1933-1943. doi: 10.2147/JMDH.S377319. PMID: 36072279; PMCID: PMC9442910.
5. Daniel G., Abebe K., Gebeyehu B., Hyder U., Kunuz H., Selass K., **Sileshi D.**, Getu D. (2021) Prevalence and Predictors of Contraceptive Use Among Women of Premenopausal Period in Ethiopia: A Retrospective Cross-Sectional Data Analysis *Journal of Contraception* 196.191.116.189 on 31-Aug-2021
6. Tsegaye, D., Shuremu, M., Oljira, D., **Dubale, S.**, Befekadu, G., & Bidira, K. (2021). COVID – 19 related knowledge and preventive practices early in the outbreak among health care workers in selected public health facilities of Illu aba Bor and Buno Bedelle zones, Southwest Ethiopia. *BMC Infectious Diseases*, 21(1), 490. <https://doi.org/10.1186/s12879-021-06218-0>
7. Asefa W, **Dubale S** (2016) Anti Malaria Drug Utilization Pattern in Selected Malarious Areas of Ilu Aba Bora Zone Health Facilities, Oromiya Regional State, South West Ethiopia. *J Drug Res Dev* 2(2): doi <http://dx.doi.org/10.16966/2470-1009.114>
8. **Sileshi Dubale** Tsegaye Berkessa Dereje Oljira . Quality and Treatment Outcomes of Directly Observed Treatment of Short-Coarse of Tuberculosis (DOTS) in South West Ethiopian: A cohort study. *Indo American Journal of Pharmaceutical Research*.2017:7(02).
9. Dame T, **Sileshi D**. Current Knowledge of Tb Patients on Initiation of Treatment in Mettu Town. *BJSTRMS.ID.001709*. DOI: 10.2671/BJSTR.2018.08.001709
10. **Sileshi D**, Efrem N, Muluneh S . “Treatment Outcomes and Determinant Factors of Under- Five Children with Malnutrition Treated in Ilu Aba Bor Zone Health Facilities, South West Ethiopia”. *Biomed J Sci & Tech Res* 20(2)-2019. *BJSTR. MS.ID.003425*.
11. **Dubale S**, Suleman S, Gurmesa A (2017) Failure Mode and Effect Analysis (FMEA) of IV-Medication Process in Mettu Karl Hospital, Mettu town, Oromiya Regional State, South West Ethiopia 3(1): doi <http://dx.doi.org/10.16966/2469-6714.118> (Msc. thesis)

### Lists of PhD Candidate's Training and Certificates

PhD Student Name: **Sileshi dubale**

Student ID Number: **RP 0158/10**

Admission modality of the PhD candidate: **A1 Category Registered**

Department: **Pharmacy**

Program: PhD in **Pharmaceutical Sciences**

| <b>Study program</b>                                                                                                                                                                                                                                                                       | <b>Cr. hrs.</b> | <b>ECTS</b> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-------------|
| <b>Common courses</b>                                                                                                                                                                                                                                                                      |                 |             |
| Systematic Review and Meta-Analysys, Synthesis                                                                                                                                                                                                                                             | 1               | 1.5         |
| Systematic writing and Communication                                                                                                                                                                                                                                                       | 1               | 1.5         |
| Advanced Biostastics                                                                                                                                                                                                                                                                       | 2               | 3           |
| Health care and Research Ethics                                                                                                                                                                                                                                                            | 1               | 1.5         |
| Philosophy of Science                                                                                                                                                                                                                                                                      | 1               | 1.5         |
| Advanced Research Method                                                                                                                                                                                                                                                                   | 3               | 5           |
| <b>Specialist courses</b>                                                                                                                                                                                                                                                                  |                 |             |
| Chemometrics in Pharmaceutical Science                                                                                                                                                                                                                                                     | 2               | 3           |
| Prenciples of Drug Discovery and Development                                                                                                                                                                                                                                               | 4               | 6           |
| Pharmaceutical Formulation Development and Process Analytical Technology                                                                                                                                                                                                                   | 4               | 6           |
| Analytical and Bioanalytical Methods                                                                                                                                                                                                                                                       | 3               | 5           |
| Molecular Pharmacology                                                                                                                                                                                                                                                                     | 2               | 3           |
| New Trends in Herbal Medicines                                                                                                                                                                                                                                                             | 2               | 3           |
| PhD Proposal Development & Dissertation Seminar (Proposal Defense)                                                                                                                                                                                                                         |                 |             |
| Sub-total                                                                                                                                                                                                                                                                                  | 26              | 40          |
| <b>Transferable Skills</b>                                                                                                                                                                                                                                                                 | <b>Duration</b> | <b>ECTS</b> |
| Advanced training of In-vitro Antimicrobial Activity Test technique, Good laboratory and God Manufacturing manufacturing practices, principles of clinical trial, Natural product anti-cancer activity study and basic principles of LC-MS &NMR analysis at Yaoundé I University, Cameroon | 160 hr          | 6           |
| Principles of Grant Writing                                                                                                                                                                                                                                                                | 24hr            | 1           |
| Total                                                                                                                                                                                                                                                                                      |                 | 7           |
| <b>Credits for the different learning activities</b>                                                                                                                                                                                                                                       | <b>Number</b>   | <b>ECTS</b> |
| Academic support primary advisor for MSc/MPH students)                                                                                                                                                                                                                                     |                 |             |
| Main advisor for more 5 MPH students(3 at Mattu University and 2 at Jimma University)                                                                                                                                                                                                      | 15              | 25          |
| Offered different courses                                                                                                                                                                                                                                                                  |                 |             |
| Nursing pharmacology                                                                                                                                                                                                                                                                       | 4               | 7           |
| Research Methodology                                                                                                                                                                                                                                                                       | 3               | 5           |
| Deffernt undargradute course fo pharmacy and health students                                                                                                                                                                                                                               | varies          |             |
| Sub-total                                                                                                                                                                                                                                                                                  |                 | 39          |

|                                                                                                                                                                                                                                                                                                                                                                                                                                              |         |          |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----------|
| <b>Conference presentation/Attendance</b>                                                                                                                                                                                                                                                                                                                                                                                                    |         |          |
| <i>Abstract submission and a poster presentation on “ Bioassay-guided isolation and structural elucidation, antimicrobial compounds from Crinum abyssinicum root bulb (Amaryllidaceae) on national research conference organized by Ethiopia Public Health institute on December 8-10.</i>                                                                                                                                                   |         | 2        |
| <i>Paper submission and oral presentation on “ Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia” the National conference organized by Wpllega University on 20and 21March 2022 at main campus Nekemte</i>                                                                                                                                                                              |         | 3        |
| <i>Paper submission and oral presentation on “Treatments of infections using Ethnomedicine and indigenous knowledge in Southwest Ethiopia, Organized by Mattu University at 6th annual research conference which was held from May 7-8 2021</i>                                                                                                                                                                                              |         | 3        |
| <b>Sub-total</b>                                                                                                                                                                                                                                                                                                                                                                                                                             |         | <b>8</b> |
| <b>Scientific publications after PhD erolment</b>                                                                                                                                                                                                                                                                                                                                                                                            | Journal | ECTS     |
| <b>Dubale, S.</b> , Abdissa, N., Kebebe, D., Debella, A., Zeynudin, A., & Suleman, S. (2023). Ethnomedicinal plants and associated indigenous knowledge for the treatment of different infectious diseases in Ethiopia. Journal of Herbal Medicine, 40(May), 100669. <a href="https://doi.org/10.1016/j.hermed.2023.100669">https://doi.org/10.1016/j.hermed.2023.100669</a>                                                                 | A1      | 5        |
| <b>Dubale S</b> , Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. Journal of Experimental Pharmacology.                                                                                                                                                                                                                                  | A1      | 5        |
| Jifar WW, Geneti GB, <b>Dinssa SD</b> . The Impact of COVID-19 on Pharmaceutical Shortages and Supply Disruptions for Non-Communicable Diseases Among Public Hospitals of South West, Oromia, Ethiopia. J Multidiscip Healthc. 2022 Aug 31;15:1933-1943. doi: 10.2147/JMDH.S377319. PMID: 36072279; PMCID: PMC9442910.                                                                                                                       | A1      | 5        |
| Daniel G.,Abebe K.,Gebeyehu B.,Hyder U.,Kunuz H.,Selass K., <b>Sileshi D.</b> ,Getu D.(2021) Prevalence and Predictors of Contraceptive Use Among Women of Premenopausal Period in Ethiopia: A Retrospective Cross-Sectional Data Analysis Journal of Contraception 196.191.116.189 on 31-Aug-2021                                                                                                                                           | A1      | 5        |
| Tsegaye, D., Shuremu, M., Oljira, D., <b>Dubale, S.</b> , Befekadu, G., & Bidira, K. (2021). COVID – 19 related knowledge and preventive practices early in the outbreak among health care workers in selected public health facilities of Illu aba Bor and Buno Bedelle zones, Southwest Ethiopia. BMC Infectious Diseases, 21(1), 490. <a href="https://doi.org/10.1186/s12879-021-06218-0">https://doi.org/10.1186/s12879-021-06218-0</a> | A1      | 5        |
| Tsegaye D, Bacha L, <b>Bidira K</b> , Determinants of acute malnutrition among Children aged 6 - 59 months attending public health facilities in Illu Aba Bor Zone, South West Ethiopia: A Case Control Study. International Journal of Pediatric Nursing. 5(1), 2019                                                                                                                                                                        | A1      | 5        |
| <b>Sub total</b>                                                                                                                                                                                                                                                                                                                                                                                                                             |         | 30       |
| <b>The defense of the doctorate (Dissertation)</b>                                                                                                                                                                                                                                                                                                                                                                                           |         |          |
| Ground Total                                                                                                                                                                                                                                                                                                                                                                                                                                 |         | 114      |

Conversion factors: 1 Cr. hr = 1.67 ECTS (e.g. 3cr.hr\*1.67 ECTS= 5 ECTS); 1 ECTS = 25 hours (e.g. 1 ECTS\*40 hours/25 hours= 1.6).

**PhD Dissertation:** Ethnomedicinal Plants for Infectious Diseases in Ethiopia: Traditional Use, Antimicrobial & Antibiotic Modulating Effects, Phytochemistry, and Regulatory Perspectives

PhD candidate's Conference Report [Presentation Certificate 3]

Name of PhD-Candidate: Sileshi Dubale Dinssa

Title of the conference attended: 6<sup>th</sup> National research conference of Mattu University:  
‘Blenging Reserch and Indegenous knowledge to ensure Socio-Economic Transformation of the Nation”

Location of the conference: Mattu University, Mattu , Ethiopia

Date of the conference: 10-11 May, 2022.

Official website of the conference: <https://meu.edu.et/>

Estimated number of participants at the conference: 150

|                                              |               |   |
|----------------------------------------------|---------------|---|
| Orientation of Conference<br>(please mark x) | Local         | - |
|                                              | National      | X |
|                                              | International | - |

Please use this Conference Report Template to report to Postgraduate and Research Director of Institute of Health your participation at a conference. Each student is expected to participate at a conference of personal choice, with a total duration of a minimum of four full days. If several workshops are visited in order to come up with the required minimum of four full days, then one conference report needs to be written for each of the workshops. At least one of the conference participation should be active (as organizer, presenter or panelist). Participation is at each student's own expense.

Note:

- The report should be approved by all supervisors
- Conference report should be of minimum of 500 words, maximum 1000 words.
- The report should be delivered no later than 14 days after the end of the workshop.

- The report should be submitted to Postgraduate and Research Director of Institute of Health in two copies.
- The report should be accompanied with evidence for participation of the conference as either certificate of participation or official letter from organizer.

Please outline the topic, the scope and intention of the conference. Who is the organizer of the conference(*word count 200*)

Wollega University, organized the national research conference at main campus, Nekemte. The conference theme was stated as “ Research Technology and Innovation for Resilience and Development in changing Environment: Different research findings from different fields of study were presented in different sections with the sub-themes of ‘ STEM, Agriculture and Food Security , Health and Medicine, Education and Biodiversity Conservation, Ecotourism and Hospitality Management

Please give some information on the other speakers and their topics of the conference. You need only to refer to those parts that you actually attended to (*word count 100*)

Fifteen studies were presented that focused on chronic and non-communicable diseases, infectious diseases, antimicrobial resistance, diagnostic tools, and ethnomedicinal-related topics. Most of them focused on interventional studies that focused on sustainable development goals and had a tight relationship with the research theme. As part of the presentation, the use of traditional medicine and medicinal plants was discussed for enhancement of health promotion, health prevention, and integration of TM with MM in primary health care. It was a very interactive presentation in which the scientific communities exchanged their knowledge, which helped us with our further studies.

Please outline the composition of the audience: approximate number, countries of origin, backgrounds (*word count 100*)

Totally, about 1300 participants were involved, and 165 of them were from the sub-team of health and medicine. There were participants who submitted their papers online from Europ, and the last were from Ethiopia; a total of more than 65 research findings were presented from different parts of the country, focusing on the theme of the conference.

Please outline in which way you have participated in the conference (eg poster presentation, Talk). Please report on how your participation went, what the reaction in the audience was like (*word count 300*)

The presenter PhD candidate) presented an oral presentation. The participants raised different questions related to the topic, added their comments, and suggested recommendations. Most of the questions were

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |           |       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|-------|
| <p>about the need for explanations on how to enhance medicinal plant research. The critical questions were on how to identify traditional medicinal practitioners since they practice it in their own homes and how to get full information on the therapeutic claims of medicinal places.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |           |       |
| <p>Please outline the topic and content of what you presented. Please explain even if you assume that your project is known (<i>Word count 200</i>)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |           |       |
| <p>I presented a research finding in titled: On the research paper titled “Phytochemical Screening and Antimicrobial Activity <i>Evaluation of Selected Medicinal Plants in Ethiopia</i>”</p> <p>The study was presented by outlining the goal of the study, which is to perform phytochemical screening and antimicrobial activity on selected medicinal plants. Accordingly, flavonoids, alkaloids, and phenols were the most numerous phytochemical components discovered. The crude extracts and chloroform fractions of the extracts had the highest activity against the tested microorganisms. The crude extract of <i>Thalictrum rhynchocarpum</i> Quart.-Dill. &amp; A.Rich root showed outstanding efficacy against all investigated bacteria, with the lowest minimum inhibitory doses of 0.48 µg/ml against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>, surpassing the reference medications. The antibacterial plant extract has a promising fraction.</p> |           |       |
| <p>Please explain whether and how the participation was useful for you and your future career (<i>Word count 100</i>).</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |           |       |
| <p>In the conference, the role of scientific Research Technology and Innovation for Resilience and Development in changing Environment:. During my presentation, the participants addressed the value of medicinal plants and indigenous knowledge in the treatment of human and animal ailments, as well as economic growth in their own nations. Various researchers expressed their opinions and insights on the significance of traditional medicinal plant composition and the necessity for research to focus on the integration of TM and MM in primary health care. For me, it was a fantastic opportunity to get feedback on the results from other participants.</p>                                                                                                                                                                                                                                                                                                        |           |       |
| Name                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Signature | Date  |
| PhD student: <u>Sileshi Dubale Dinssa</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           | _____ |



## **Anexs**

### **Annex -1. English and Afaan Oromo Vertion of Questioners**

#### English Version Questionnaire Format

Jimma University, Institute of Health Department of Pharmacy

Participant information sheet and consent form (English version

Part I: participant Information Sheet

I -----the undersigned is PhD student in pharmaceutical science at Jimma University pharmacy department. And working research entitled “,Ethnomedicinal Plants for Infectious Diseases in Ethiopia: Traditional Use, Antimicrobial & Antibiotic Modulating Effects, Phytochemistry, and Regulatory Perspectives

” I agree to accept responsibility for the scientific, ethical, and technical conduct of the research project through coordinative efforts of all research groups for provision of progress reports as per the term, submission of technical reports and scientific publications to Jimma University if grant is awarded as a result of this application. The feedback of the result will be communicated to the study areas and to the practitioners. Name of principal investigator Sileshi Dubale Signature of the principal investigators Sig.\_\_\_\_\_Date\_\_\_\_\_

Study area- South west Ethiopia (Special focus of Ilu Aba Bor)

Research fund- by Jimma University and other

Research objective: The main aim of this project is to identify and document potential traditional medicinal plants and to isolate compounds with anti-microbial activities from some selected Ethiopian traditional medicinal plants. The purpose of the study: The purpose of the this study is to gather information and document on ethnomedicinal information from Ilu Aba Bor zone districts for prioritization of potential medicinal plants and for conducting of antimicrobial activity test, synergistic evaluation and phytochemical analysis. It focuses on gathering and documenting of traditional medicinal plants and the associated ethno-medicinal knowledge, anti-microbial activity test, phytomedicinal investigation and to evaluate synergic effect of selected medicinal plants with conventional antibiotics on resistant pathogen. This is believed to add up to the country's database of medicinal plants and in documenting indigenous knowledge of the people to discover cost effective antimicrobial lead compound and

it may lay down the base for “re-using” of the conventional antibiotics with in short period of time with low development cost with minimal side effect

Responsibilities of the practitioners: Voluntarily to divulge full information on their knowledge and indigenous interpretation of infection, their perception and explanation about different infections, medicinal plants used for the infectious diseases, plant parts used, mode of administration, growth habit, preparation methods, and season of collection as well as to show the reported medicinal plants genuinely.

Study procedure: Data will filled by self-administered interview and data collectors

Risk: No risk , it will take 25-30 minutes of your time.

Benefit: The study is beneficial for documentation of ethnomedicine knowledge and improvement of Traditional Medicine utilizations in Ethiopia

Confidentiality: The study is not included participant name

Whom to contact: for any incontinency and doubt please contact

Sileshi Dubale phone +251 917806080, email [sileshi.dubale@gmail.com](mailto:sileshi.dubale@gmail.com)

Part II: Assurance of the principal investigator

Greetings, how are you doing? My name is ----- and my colleague's name is ----- We are data collectors for the study, which intends to survey medicinal plants used for infectious diseases and any practices related to them. You were purposefully selected to be part of this survey, and that is why we would like to interview you. If you are willing to participate in this study, you are kindly invited to provide the honest information required of you. We would like to get information on your knowledge of traditional medicine as well as your practice of indigenous or traditional medicine. Your participation in this study is voluntary, and we will not offer any incentives. You do not need to write your name; hence, your response will not be linked to your identity. Your participation and the data you provide will be kept completely confidential. You can refuse to participate or decline the interview at any time. We would like to greatly appreciate your enthusiastic support in responding to the questionnaire by yourself. Do you agree to voluntarily participate in this study?

Yes \_\_\_ No\_\_\_ If yes, sign ----- date -----

We thank you for consenting to participate in this study. Consent to participate in the study

I have received the relevant information about the study and agreed to participate in it without coercion or undue influence.

Signature: \_\_\_\_\_ Name of data collector: \_\_\_\_\_ Date: \_\_\_\_\_

Address District/Kebele/Got: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_

Section I :Ethnomedicinal survey and documentation of knowledge, semi-structured ( Questionnaire 001)

Woreda /District      
 Kebele      
 Local residence name /Got      
 Interviewer Identification      
 Questionnaire identification No.

| Step 1 General information on respondents |                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                          |
|-------------------------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|
| No                                        | Questions                                                                                  | Response with Code                                                                                                                                                                                                                                                                                                                                                                                                                                | Site tailoring                                           |
| 1                                         | Gender                                                                                     | 1. Male <input type="checkbox"/><br>2. Female <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                            |                                                          |
| 2                                         | Age                                                                                        | Years -----                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                          |
| 3                                         | Religion                                                                                   | 1. Orthodox <input type="checkbox"/><br>2. Catholic <input type="checkbox"/><br>3. Protestant <input type="checkbox"/><br>4. Muslim <input type="checkbox"/><br>5 Wakefata <input type="checkbox"/><br>6. Other <input type="checkbox"/> Please specify -----                                                                                                                                                                                     |                                                          |
| 4                                         | Ethnicity                                                                                  | -----                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                          |
| 5                                         | Address                                                                                    | Urban <input type="checkbox"/> Rural <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                     |                                                          |
| 6                                         | How long you have lived in the area                                                        | -----                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                          |
| 7                                         | Marital status                                                                             | 1. Single <input type="checkbox"/> 2. Married <input type="checkbox"/> 3. Widowed <input type="checkbox"/> 4. Divorced <input type="checkbox"/>                                                                                                                                                                                                                                                                                                   |                                                          |
| 8                                         | Education status                                                                           | 1. Not read and write <input type="checkbox"/> 2. Read only <input type="checkbox"/> 3. Read and write <input type="checkbox"/><br>4. Elementary school <input type="checkbox"/> 5. Secondary school <input type="checkbox"/> 6. College/university <input type="checkbox"/>                                                                                                                                                                      |                                                          |
| 9                                         | How long have you been practicing as traditional healer?                                   | -----                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                          |
| 10                                        | How did you acquire your knowledge and wisdom of healing for traditional medicine?         | 1. From family (grandfather, father, mother, etc) <input type="checkbox"/> 2. From other traditional healer(s) while working as an learner <input type="checkbox"/><br>3. Learning from traditional medicine books & observation <input type="checkbox"/><br>4. Learning from religious institutions <input type="checkbox"/><br>5. A gift given from God <input type="checkbox"/> 6. Other sources <input type="checkbox"/> Please specify ----- |                                                          |
| 11                                        | Did you transfer your knowledge to other person                                            | 1. Yes <input type="checkbox"/><br>2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                 | If the response is No, please go and ask question No. 13 |
| 12                                        | To whom you transfer your knowledge? More than two answer is possible                      | 1.To your children only <input type="checkbox"/> 2.To relatives <input type="checkbox"/><br>3.To any person who need it <input type="checkbox"/> 4.Not at all <input type="checkbox"/><br>5.Other <input type="checkbox"/> specify -----                                                                                                                                                                                                          |                                                          |
| 13                                        | Do you think young generation have willing to get information on Traditional Medicine>     | 1. Yes <input type="checkbox"/><br>2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                 |                                                          |
| 14                                        | If your answer is No what do you think about is reason (More than two answers are possible | Thinking TM as Back ward practice <input type="checkbox"/><br>Thinking TM as harmful practice <input type="checkbox"/><br>No reason <input type="checkbox"/> Other <input type="checkbox"/> specify -----                                                                                                                                                                                                                                         |                                                          |

|    |                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                         |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 15 | In which area of traditional medicine do you serve the community? (Note:- more than one response may be possible)                                                      | 1. Herbalist (using medicinal plants only) <input type="checkbox"/><br>2. Spiritual healer (Debtera) <input type="checkbox"/><br>3. Bone settler (Wegesha) <input type="checkbox"/><br>4. Capping/koba 5. Other <input type="checkbox"/> Please specify -----                                                                                                                           |
| 16 | Which types of traditional medicine remedies or approach do you employ? (Note:- more than one response may be possible)                                                | 1. Plants and plant products <input type="checkbox"/> 2. Animal products <input type="checkbox"/><br>3. Minerals <input type="checkbox"/> 4. Holy water (Tsebel Christians)/Zemzem Muslim) <input type="checkbox"/><br>5. Others <input type="checkbox"/> Please specify -----                                                                                                          |
| 14 | What are the most common infectious diseases in your area?                                                                                                             | 1. -----2. -----3. -----<br>4. -----5. -----                                                                                                                                                                                                                                                                                                                                            |
| 15 | What are the most 10 plants you use for infectious diseases                                                                                                            | 1. -----2. -----3. -----<br>4. -----5. -----6. -----<br>7. -----8. -----9. -----10. -----                                                                                                                                                                                                                                                                                               |
| 16 | What are the major threats to medicinal plants that you have used?                                                                                                     | 1. Deforestation for agriculture <input type="checkbox"/> 2. Fire <input type="checkbox"/><br>3. Massive harvest and over utilization of medicinal plants from forests and other uncultivated areas <input type="checkbox"/> 4. Other <input type="checkbox"/> Please specify -----                                                                                                     |
| 17 | What are the traditional practices used by the local community for medicinal plant conservation? or how do you conserve and manage endangered medicinal plant species? | 1. Cultivation of medicinal plants in home garden <input type="checkbox"/><br>2. Guarding against deforestation and fire <input type="checkbox"/><br>3. Control of massive harvest of wild medicinal plants <input type="checkbox"/><br>4. Planting and conservation <input type="checkbox"/> 5. Doing Nothing <input type="checkbox"/> 6. Other <input type="checkbox"/> specify ----- |
| 18 | How do you see accessibility of medicinal plants when compared with the past Decade or before?                                                                         | 1. Easily accessed since I collect from home gardens <input type="checkbox"/><br>2. Accessed from forests without much problems <input type="checkbox"/><br>3. Difficult to access due to deforestation and agricultural expansion <input type="checkbox"/><br>4. Other <input type="checkbox"/> Please specify -----                                                                   |
| 19 | What others uses have the traditional medicinal plants that you use for illness?                                                                                       | 1. They are also used as food <input type="checkbox"/> 2. They are also used as spices <input type="checkbox"/><br>3. They are also used for cosmetic purpose <input type="checkbox"/> 4. Other <input type="checkbox"/> Please specify -----                                                                                                                                           |
| 20 | Do you believe that the remedies employed in traditional medicine should be documented and scientifically evaluated for their safety and efficacy through research?    | 1. Yes <input type="checkbox"/><br>2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                       |
| 21 | Do you have collaboration with other traditional healers?                                                                                                              | 1. Yes <input type="checkbox"/> 2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                          |
| 22 | If yes, what are the most common means of collaborations with other traditional healer?                                                                                | 1. Information and experience sharing on knowledge & practice <input type="checkbox"/><br>2. Sharing of traditional medicinal plants or remedies <input type="checkbox"/><br>3. Others <input type="checkbox"/> 4. Please specify -----                                                                                                                                                 |

| Step 2 Information on traditional health care service |                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                |
|-------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| No                                                    | Questions                                                                                                                                                                                                                          | Response with Code                                                                                                                                                                                                                                                                                                                                                                                                                                            | Site tailoring |
| 23                                                    | From where do you get the remedies (sources of medicinal plants) ?<br>Note: more than one answer is possible)                                                                                                                      | 1. Home garden (cultivated plants) <input type="checkbox"/><br>2. Collection from local forests or fields (wild collection) <input type="checkbox"/><br>3. From traditional medicine distributors & vendors in markets <input type="checkbox"/><br>4. Other sources <input type="checkbox"/> please specify -----                                                                                                                                             |                |
| 24                                                    | Do you keep records of clients (name, age, sex, illness history, diagnosis, remedy given, etc.) obtaining treatment at your traditional medicines units ?                                                                          | 1. Yes <input type="checkbox"/> 2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                                |                |
| 25                                                    | How do you keep/store the traditional remedies that are to be dispensed for clients?                                                                                                                                               | 1. Wrapping in plastics, paper bags or newspaper <input type="checkbox"/><br>2. In containers that have lids with labels <input type="checkbox"/><br>3. Others <input type="checkbox"/> please specify -----                                                                                                                                                                                                                                                  |                |
| 26                                                    | Do you believe that the remedies employed in traditional medicine should be documented and evaluated through research for their safety and efficacy?                                                                               | 1. Yes <input type="checkbox"/> 2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                                |                |
| 27                                                    | If yes, are you willing to reveal the secrets of traditional medicine knowledge and collaborate with researchers for safety and efficacy evaluation of the remedies you prescribe to clients?                                      | 1. Yes <input type="checkbox"/> 2. No <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                                |                |
| 28                                                    | If No, could you please explain as to why you are not willing to reveal the secrets of traditional medicine ?                                                                                                                      | 1. Because if secrecy is revealed TM will not be effective <input type="checkbox"/><br>2. TM is my means of livelihood (income source) <input type="checkbox"/><br>3. There is no right for royalty or beneficence to reveal the secrecy <input type="checkbox"/> 4. I have a promise from my ancestors who taught me TM knowledge not to reveal the secrecy <input type="checkbox"/> 5. Other reasons <input type="checkbox"/> please specify -----          |                |
| 29                                                    | Do you think that traditional medicine has some drawbacks or weak sides?                                                                                                                                                           | 1. Yes, TM has some negative effect <input type="checkbox"/><br>2. No, TM has no any weak side <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                       |                |
| 30                                                    | If Yes, which of the following could be the reasons                                                                                                                                                                                | 1. Problem in the diagnosis of illness <input type="checkbox"/> 2. Use of intangible aspects, sorcery, etc <input type="checkbox"/><br>3. Problems in dosage level of traditional remedies <input type="checkbox"/><br>4. Harmful practices such as tooth extraction, uveloctomy, etc <input type="checkbox"/><br>4. Other reasons <input type="checkbox"/> please specify -----                                                                              |                |
| 31                                                    | How much do you charge your clients for your service and traditional remedies?<br>(Note:- more than one response may be possible)                                                                                                  | 1. Free of charge for those who could not pay <input type="checkbox"/> 2. 1 Birr to 50 Birr <input type="checkbox"/><br>3. 51 Birr to 100 Birr <input type="checkbox"/> 4. More than 100 Birr <input type="checkbox"/><br>5. No fixed cost, depending on the economic status of the client <input type="checkbox"/><br>6. Payment in kind (e.g. .livestock, grain, food, etc) <input type="checkbox"/> 7. Other <input type="checkbox"/> please specify ----- |                |
| 32                                                    | What are the opinions reflected by your clients after taking the traditional remedies for the illness ?                                                                                                                            | 1 Cured/improved from illness <input type="checkbox"/> 2 Partially improved from illness <input type="checkbox"/><br>3. Not cured or improved from illness <input type="checkbox"/> 4. Other reasons <input type="checkbox"/> please specify -<br>-----                                                                                                                                                                                                       |                |
| 33                                                    | If the response is yes, express your appreciation for him by saying, Thank you for your sincere support and then ask, Please give me Information's details on traditional medicines used for treating human and livestock diseases | Please use the Tables 1 and 2 below to write responses                                                                                                                                                                                                                                                                                                                                                                                                        |                |

Table 1: Detailed information's on traditional medicines used by the traditional healer for treating Neglected Tropical Diseases

| No | Plant name |                 | Disease used for | Part used | How to Prepare | Dosage form | Routes of administration | Expected side effect | antidote of treatment | Amount to be taken | Appropriate time of collection | Grow form (tree, shrub, parasite) | Environmental impact(endangered Not endangered) | Voucher number |
|----|------------|-----------------|------------------|-----------|----------------|-------------|--------------------------|----------------------|-----------------------|--------------------|--------------------------------|-----------------------------------|-------------------------------------------------|----------------|
|    | Local name | Scientific name | Family           |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 1  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 2  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 3  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 4  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 5  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 6  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 7  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 8  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 9  |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |
| 10 |            |                 |                  |           |                |             |                          |                      |                       |                    |                                |                                   |                                                 |                |

## Annex I.II. Afaan Oromoo Version of the Questioner Format

### A. Mirkaneessa Qorataa dura Bu'aa

Anii maqaan koo armaan gaditti kan ibsame qoanoo kana keessat itti gaafatamumaa koo kanan fudhu dha. Teknikaa fi haala naamusa qoranoofi qo'ano eeggateen hojachuuf, gabasa isaa yeroo yerootti dhiyeesuuf, fi bu'aa qoranichaa maxansee University Jimmaatti dhiyeesuufi dhigamakoo ba'uufi nan beekisisa.

Maqaa qo.ataa dura bu'aa \_\_\_\_\_ Mallattoo \_\_\_\_\_ Guya \_\_\_\_\_

Bakka Qoanaa Godina Ilu Abaa Boor

Qoannicha malaqaan kan deeggaru – Univarsitii Jimmaa tiif dhaabbatoota adda adaa

Kayoo qoranichaa: Kaayyoo goeroon qoanichaa:- qorichoota aadaa barumsa uumamatin fayadamanu adda baasanii galmesuu, keemikalaa biqiltuu qoricha aadaa keesa jiru addabasanii ilbisota dhukkuba daddarboofidanuufi kan ijaan hin argamne irratti yaalii geggesuu dha.

Mala qoranichaa : Odeeffanoo mala af-gaafitiin geggefama.

Hameenya : Qoanich ammeenya namaraan ga'u hin qabu Yerokee daqiiqaa 25-30 qofa fudhata/

Fayidaa: Qoanoo kuni qorchalee adaa beekumsa umamatiin qaratani addabasuu fi gara fuulduratti qoricha aadaa aoricha ammayaa waliin sirna fayaa bu'uraa waliin akkata fayadamuun danda'amu bu'uura kaa'uuf gargaara.

Iccitii: icitii eeguufi maqaan nama gaafatamuu hin barreffamu, oddeffanonis enyutuu hin imamu.

Yoo shakiin jiraate teessoo armaan gadiitiin naqunnamuu dandeessu

- Sileshi Dubale phone +251 917806080, email [sileshi.dubale@gmail.com](mailto:sileshi.dubale@gmail.com)

### B) Shiitii odeffanoo irmaatootaa

Akkam jirtu ?, ani maqaan koo ----- n jedhama maqaan iryaa kootiimoo --  
----- jedhama. Nutii qo'ano matadureen issaa “ Bikiltuu qorichoota adaa dhukkuba dadarbootii fayyanu kan jedhuufi barnnoota humamaa wal fakkatanu odeffachuu kan dhufneedha. Isin akka bekaa garee qo'annoo kanaa akka taatanuufi kan filamtanii dha. Yoo fedha keessan ta'ee odeffanoo ga'aa ta'ee akka nuu kenitanuufi kabajaan isin gaafana. As keessatti irmatanii odeffanoo ga'aa nuu kennuun keessan fedhidhaan waan ta'eef kaffaltii tokko ilee hin qabu. Maqaa keessan barresuun hinbarbachiiisu icitiin isin kennitanusii namati

hin imamu. Irmachuu diduufis mirgga gutuu qabdu. irmachuufi fedhii qabduu? Eyee \_\_\_\_\_ waa'uu \_\_\_\_\_

Eyee yoo jettan mallattoo \_\_\_\_\_ Guyaa \_\_\_\_\_

Irmanakessaniifi isin galatefana

Ani odeeffataa kanan ta'e odeefanoo ga'aa kennee ubachisuu koo nan mirkaneessa

Mallattoo : \_\_\_\_\_ Maqaa odeeffataa : \_\_\_\_\_ Guyaa \_\_\_\_\_

Tesoo Anaa/ganda/Goxii/garee: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_

Kutaa Tokkoffaa : qoeicha adaatiifi galmeessa qabiyyee beekkumssa uumama ( gafii 001)

Anaa \_\_\_\_\_  
gandda \_\_\_\_\_  
Bakka \_\_\_\_\_  
Enymaa odeeffataa \_\_\_\_\_  
Enumaa gafii No. \_\_\_\_\_

| Adeemssa 1 Odeeffanoo waliigalaa |                                    |                                                                                                                                                                                                                                                                                                                                                                                                    |        |
|----------------------------------|------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| Lak.                             | Gafii                              | Debii kodii waliin                                                                                                                                                                                                                                                                                                                                                                                 | Darbii |
| 1                                | Gender                             | 1DHI <input type="checkbox"/><br>2. Dha <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                   |        |
| 2                                | Umurii                             | Wagaan -----                                                                                                                                                                                                                                                                                                                                                                                       |        |
| 3                                | Amantii                            | 1. Ortodoksii <input type="checkbox"/><br>2. Katolikii <input type="checkbox"/><br>3Protestantii <input type="checkbox"/><br>4. Muslima <input type="checkbox"/><br>5 Wakefata <input type="checkbox"/><br>6Kan biraa <input type="checkbox"/> Maloo adda basii ibisi -----                                                                                                                        |        |
| 4                                | Gosa/sanyumaa                      | -----                                                                                                                                                                                                                                                                                                                                                                                              |        |
| 5                                | Bakka teessoo                      | magalaa <input type="checkbox"/><br>Badiyaa <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                               |        |
| 6                                | Wagaa meeqa as jiraatte            | -----                                                                                                                                                                                                                                                                                                                                                                                              |        |
| 7                                | Dhimma gaa'elaa                    | 1. kan hinfuune <input type="checkbox"/><br>2. kan fuudhe <input type="checkbox"/><br>3. kan biraa du'ee <input type="checkbox"/><br>4. kan adda baa'e <input type="checkbox"/>                                                                                                                                                                                                                    |        |
| 8                                | Sadarkaa barnotaa                  | 1. Tasa barreesuufi dhubisuu hin danda'u <input type="checkbox"/><br>2. Barresuu qofa danda'a/s <input type="checkbox"/><br>3. barressuufi dubisuu kan danda'u <input type="checkbox"/><br>3. sadarkaa tokkoffaa <input type="checkbox"/><br>4. sadarkaa lammaffaa <input type="checkbox"/><br>5. Kollejii/Univarsitii <input type="checkbox"/>                                                    |        |
| 9                                | Qoricha aadaa waggaa meeqa hojette | -----                                                                                                                                                                                                                                                                                                                                                                                              |        |
| 10                               | Oogummaa kana akkamit argatte      | 1. Maatii irraa (akaakayuu, abbaa Haadha ) <input type="checkbox"/><br>2. Nama qoricha haadaa beekanu waliin hojjechuun <input type="checkbox"/><br>3. Kataaboleefi barachuu dhaan <input type="checkbox"/><br>4. Abboota amantii irraa barachuun <input type="checkbox"/><br>5. Kenaa Waaqa irraa argadhee dha <input type="checkbox"/><br>6. Kan biraa <input type="checkbox"/> Adda baasi ----- |        |



| Adeemssa 2 Odeeffanoo waa'ee qoricha aadaa |                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                  |                                                |
|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Lakk                                       | Gafii                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                         | Deebii koodii waliin                                                                                                                                                                                                                                                             | Darbii                                         |
| 11                                         | Beekumssa kee kana namoota biraatiif ni dabarsitaa                                                              |                                                                                                                                                                                                                                                                                                                                                                         | 1. Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                          | Yoo deebiin kee waa'uu ta'ee , lak. 13ti darbb |
| 12                                         | Yoo deebiin kee Eyyee ta'ee, eenyutt Oogummaa kee dabarsita?                                                    |                                                                                                                                                                                                                                                                                                                                                                         | 1.Ijoollee koo Qoffa <input type="checkbox"/> 2.Fira koo <input type="checkbox"/> 3.Namaa barbaade Unddatti <input type="checkbox"/><br>4.Enyutilee hin dabarssu <input type="checkbox"/> 5.Kan biraa <input type="checkbox"/> adda baasi -----                                  |                                                |
| 13                                         | Dhaloonni haaraan kuni oogummaa qoricha aadaa t irmachuuf fedha qaba jettee yaaddaa?                            |                                                                                                                                                                                                                                                                                                                                                                         | 1. Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                          |                                                |
| 14                                         | Yoo waa'uu jette sababiin isaa oo? (Hub: deebiin tokkoo ol ni danda'ama)                                        |                                                                                                                                                                                                                                                                                                                                                                         | Akka adaa boodatafaatti waan yaadanuufi <input type="checkbox"/><br>Akka aadaa boodatafaatti yaaduu <input type="checkbox"/><br>Sababa hin qabanu <input type="checkbox"/> 4.kan biraa <input type="checkbox"/> adda baas -----                                                  |                                                |
| 15                                         | Naannoo qoricha aadaa kamirratti huummata tajaajilta? (Hub: deebiin tokkoo ol ni danda'ama)                     |                                                                                                                                                                                                                                                                                                                                                                         | 1. Herbalist (using medicinal plants only) <input type="checkbox"/> 2. Spiritual healer (Debtera) <input type="checkbox"/> 3. Bone settler (Wegesha) <input type="checkbox"/> 4. Capping/koba <input type="checkbox"/><br>5. Other <input type="checkbox"/> Please specify ----- |                                                |
| 16                                         | Burqaan qoricha aada keetii maalii? (Hub: deebiin tokkoo ol ni danda'ama)                                       |                                                                                                                                                                                                                                                                                                                                                                         | 1. Biqilttuufi bu'aa isaa <input type="checkbox"/> 2. beellada <input type="checkbox"/> 3. Albuuda <input type="checkbox"/><br>4. Bishaan qulquluu (xabala /Zamzam <input type="checkbox"/> 5. kan biraa <input type="checkbox"/> adda baasi---                                  |                                                |
| 14                                         | Dhukkubni dadarboon naanokee bayinaan jiru maalfat?                                                             |                                                                                                                                                                                                                                                                                                                                                                         | 1. -----2. -----3. -----<br>4. -----5. -----                                                                                                                                                                                                                                     |                                                |
| 15                                         | Bikilttuu qoricha aadaa edduminaan dhukkuba daddarboofi fayyadanu 10 tarreessi                                  |                                                                                                                                                                                                                                                                                                                                                                         | 1. -----2. -----3. -----4. -----<br>5. -----6. -----7. -----<br>8. -----9. -----10. -----                                                                                                                                                                                        |                                                |
| Adeemssa 2 odeeffanoo qoricha aadaa        |                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                  |                                                |
| Lak.                                       | Gaaffii                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                         | Deebii koodii waliin                                                                                                                                                                                                                                                             | darbii                                         |
| 16                                         | Biqilttuu qorchaa aadaafi fayyadanu maalttu ballesaa jira jitta                                                 |                                                                                                                                                                                                                                                                                                                                                                         | 1. Deforestation for agriculture <input type="checkbox"/><br>2. Abiddaan gubuu <input type="checkbox"/><br>3. baayyisanii qoricha aadaatti fayyadamuu <input type="checkbox"/><br>4. kanbiraa <input type="checkbox"/> adda baasi -----                                          |                                                |
| 17                                         | Qoricha naannoo aadaa keetii akkamitti kunuunsita?                                                              | 1. Boroo manaatti kunuunsuu dhaan <input type="checkbox"/> 2. akka hin ciramneefi hin gubane eeguun <input type="checkbox"/><br>3. bayyinaan akka hin fayyadamine gochuu <input type="checkbox"/> 4. biqilttuu dhaabanii kunuunsuu dhaan <input type="checkbox"/><br>5. Homaa hin godhu <input type="checkbox"/> 6. kan biraa <input type="checkbox"/> adda baasi ----- |                                                                                                                                                                                                                                                                                  |                                                |
| 18                                         | Dhiyeessii qoricha aadaa akkamitti ilaalta yeroo darbeen yeroo walbira qabddu?                                  |                                                                                                                                                                                                                                                                                                                                                                         | 1. Oddoo dha akka salphaatti argama <input type="checkbox"/><br>2. bosona keessaa dadhabii malee argama <input type="checkbox"/><br>3. Argamuunsaa sababa falama naannotifi rakkisaa dha <input type="checkbox"/><br>4. Kan biraa <input type="checkbox"/> Adda baasi-----       |                                                |
| 19                                         | Fayidaan qoricha adaa kan biraa maalfa dha?                                                                     |                                                                                                                                                                                                                                                                                                                                                                         | 1. dabalataan nyaataafi fayyadu <input type="checkbox"/> 2. dabalataan mi'esituu dhaafi fayyadu <input type="checkbox"/> 3. dabalataan dibataafi fayyadu <input type="checkbox"/><br>4.Kan biraa <input type="checkbox"/> adda baasi -----                                       |                                                |
| 20                                         | Qorichi aadaa fayyadamnu galmaanii faiddaan angi isaaniifi miidhaan isaani qoratamuu akka gqabu jettee yaaddaa? |                                                                                                                                                                                                                                                                                                                                                                         | 1. Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                          |                                                |
| 21                                         | Namoota qoricha aadaa hojetanu kan biro waliin qindoomina qabdaa?                                               |                                                                                                                                                                                                                                                                                                                                                                         | 1. Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                          | Yoo waa'uu jedhe gara gafii 23 ffaa deemi      |
| 22                                         | Yoo eyyee jette, maaliin waliin qindoomttu ?                                                                    |                                                                                                                                                                                                                                                                                                                                                                         | 1. Odeeffanoofi Muuxxanoo wal jijjiruun <input type="checkbox"/><br>2. qoricha aadaa wal jijjiruun <input type="checkbox"/><br>3. kan biraa <input type="checkbox"/> adda baasi-----                                                                                             |                                                |
| 23                                         | Qorichaa adaa essaa argata ?<br>Note: more than one answer is possible(Deebiin tokkoo ol ni danda'ama)          |                                                                                                                                                                                                                                                                                                                                                                         | 1. Oddoo keessat kunuunsuun <input type="checkbox"/> 2.Bosona Keessaa sassabuun) <input type="checkbox"/> 3. warra gurguranu iraa bituun <input type="checkbox"/><br>4. Kan Biraa <input type="checkbox"/> adda baasi-----                                                       |                                                |
| 24                                         | Yeoo yaaltu deeffanni maamila keetii(Umuri, saala, daawwaa kenamuuf...) nigalmesita ?                           |                                                                                                                                                                                                                                                                                                                                                                         | 1. Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                          |                                                |
| 25                                         | Qoricha aadaa akkamitti Kusta?                                                                                  |                                                                                                                                                                                                                                                                                                                                                                         | 1. Warakaa .Plaastikan maree <input type="checkbox"/> 2. qodaa bilqaxii keessa kaa'ee <input type="checkbox"/> 3. kan biraa <input type="checkbox"/> adda baasi-----                                                                                                             |                                                |
| 26                                         | Qorich adaa kennamu galmaa'ee qoratamuu qaba                                                                    |                                                                                                                                                                                                                                                                                                                                                                         | Eyyee <input type="checkbox"/>                                                                                                                                                                                                                                                   |                                                |

|    |                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                          |
|----|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | jatte niyaaddaa?                                                                  | 2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                       |
| 27 | Yoo Eyyee Ta'e, Oogumma dhokisaa qorcha aadaa baastee ibssuuf fedhii qabdaa??     | Eyyee <input type="checkbox"/><br>2. Waa'uu <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                     |
| 28 | Yoo waa'uu ta'e, sababa ibsuu hin barbaanneef maalii ?                            | 1. yoo dhoksaan isaa ba'ee qorich aadaa hin hojjetu <input type="checkbox"/><br>2. madda galii kooti <input type="checkbox"/> 3. ibsuuf mirgga hin qaby <input type="checkbox"/><br>4. namni nabarsiise waada nagalcheera <input type="checkbox"/> 5. kan biraa <input type="checkbox"/> adda baasi.....                                                                                                 |
| 29 | Qorich aadaa rakkoo rakkoo bukke qaba jatee yaaddaa?                              | 1. Eyyee qaba <input type="checkbox"/><br>2. Waa'uu hin qabu <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                    |
| 30 | Yoo eyyee ta'e, sababiinsaa maalii                                                | 1. rakkoo xiinxala dhukkubaa <input type="checkbox"/> 2. rakkoo dhoksaa qabatama hin qabne <input type="checkbox"/> 3. rakoo anga keniniinsa qorchaa <input type="checkbox"/><br>4. adaa miidhaa fidanu kan akka ilkaan ijoolee basuu kkf <input type="checkbox"/><br>4. kan biraa <input type="checkbox"/> adda baasi-----                                                                              |
| 31 | Maamila kee qarshii meeqa kaffalchiifta?<br>(Hub: deebiin tokkoo ol ni danda'ama) | 1. tola omaa hin qaffalchiisu <input type="checkbox"/> 2. arshii 1 anqa qarshii 50 <input type="checkbox"/><br>3. . arshii 51 anqa qarshii 100 <input type="checkbox"/> 4. qarshii 100 ol <input type="checkbox"/><br>5. gatii dhabbataa hin qabu <input type="checkbox"/> 6. kaffaltii gosa adda adda (e.g. .orii nyaata kkf) <input type="checkbox"/> 7. kan biraa <input type="checkbox"/> ibssi----- |
| 32 | Dub-deebin maamilaa maal fakkata edda yaaltee booda ?                             | 1 fayyuu/foyya'uu isaa ibssu <input type="checkbox"/> 2 waalakaan fooyya'uu isbsu <input type="checkbox"/><br>3. hinffayine/hinfooyofne jedhanii ibsu <input type="checkbox"/> 4. kan biraa <input type="checkbox"/> adda baasi-----                                                                                                                                                                     |
| 33 | Qoricota fayyadamtu akka natti imttu kabajaan sin gaaffadha                       | Bocha 1 fi 2 armaan gadii guuti                                                                                                                                                                                                                                                                                                                                                                          |

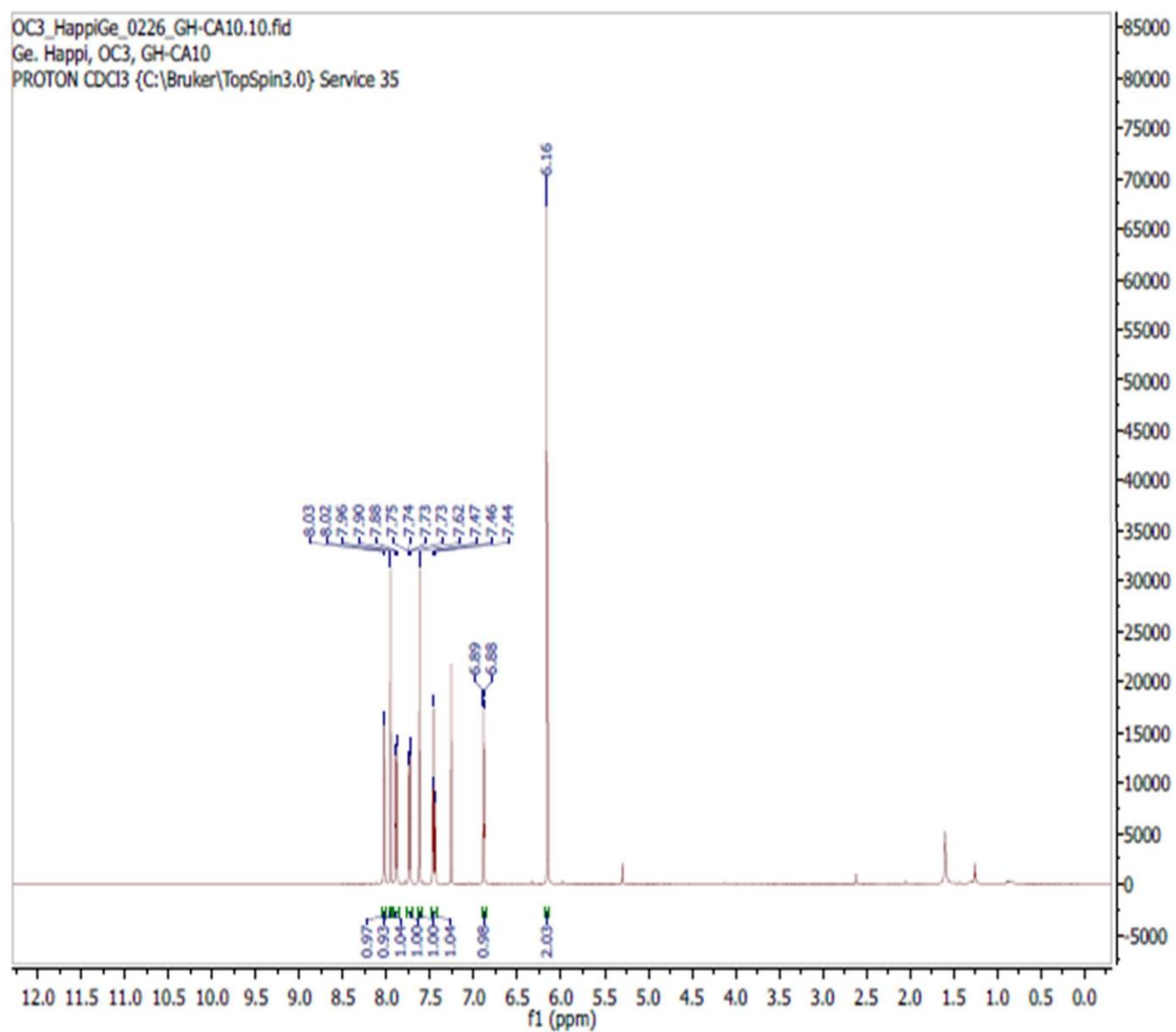
Boocha 1: odeeffan gadi fagoo waa'ee fayyadama qorcha aadaa

| lak | Maqaa qorichaa |                  |          | Dhukuba fayyadamanuuf | Qamma biqiltuu | Mala qophii isaa | bolbulamasaa | Karaa kennamu | Rakkoo bukee inni fidu | Ma alii n ittifaama | Anga fayyadamu | Waqixile e bu'atii | Yeroo bu'amu | Guddin asaa | Bakka bu'ame | Miidha irra ga'ee | Gal mee qorichaa |
|-----|----------------|------------------|----------|-----------------------|----------------|------------------|--------------|---------------|------------------------|---------------------|----------------|--------------------|--------------|-------------|--------------|-------------------|------------------|
|     | Maqaa naanoo   | Maqaa sayinsawaa | matiisaa |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 1   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 2   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 3   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 4   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 5   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 6   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 7   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 8   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 9   |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |
| 10  |                |                  |          |                       |                |                  |              |               |                        |                     |                |                    |              |             |              |                   |                  |

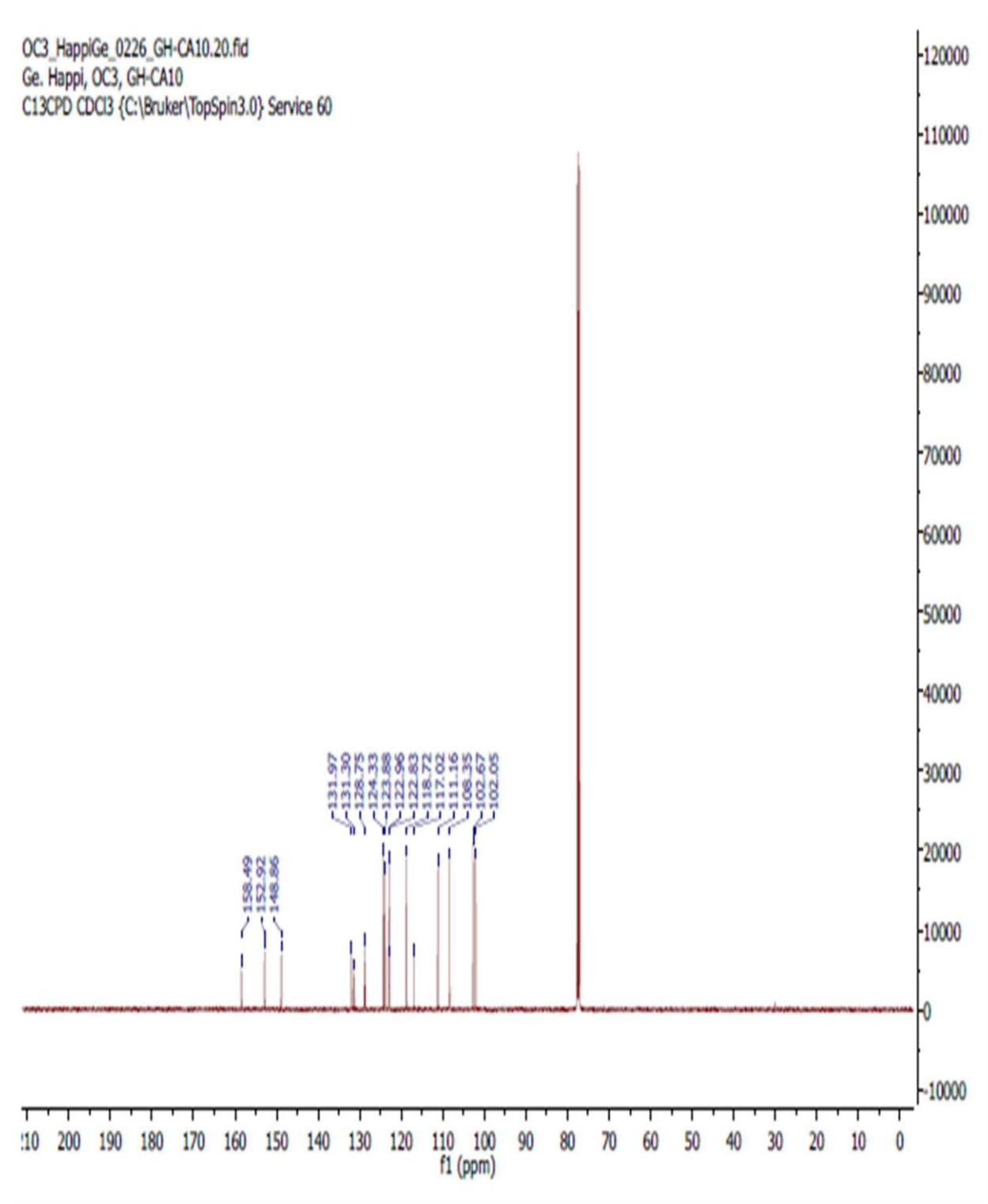
## Annex 2 NMR Spectras

### Annex 2.1 NMR Spectras of Ca 10 Compound

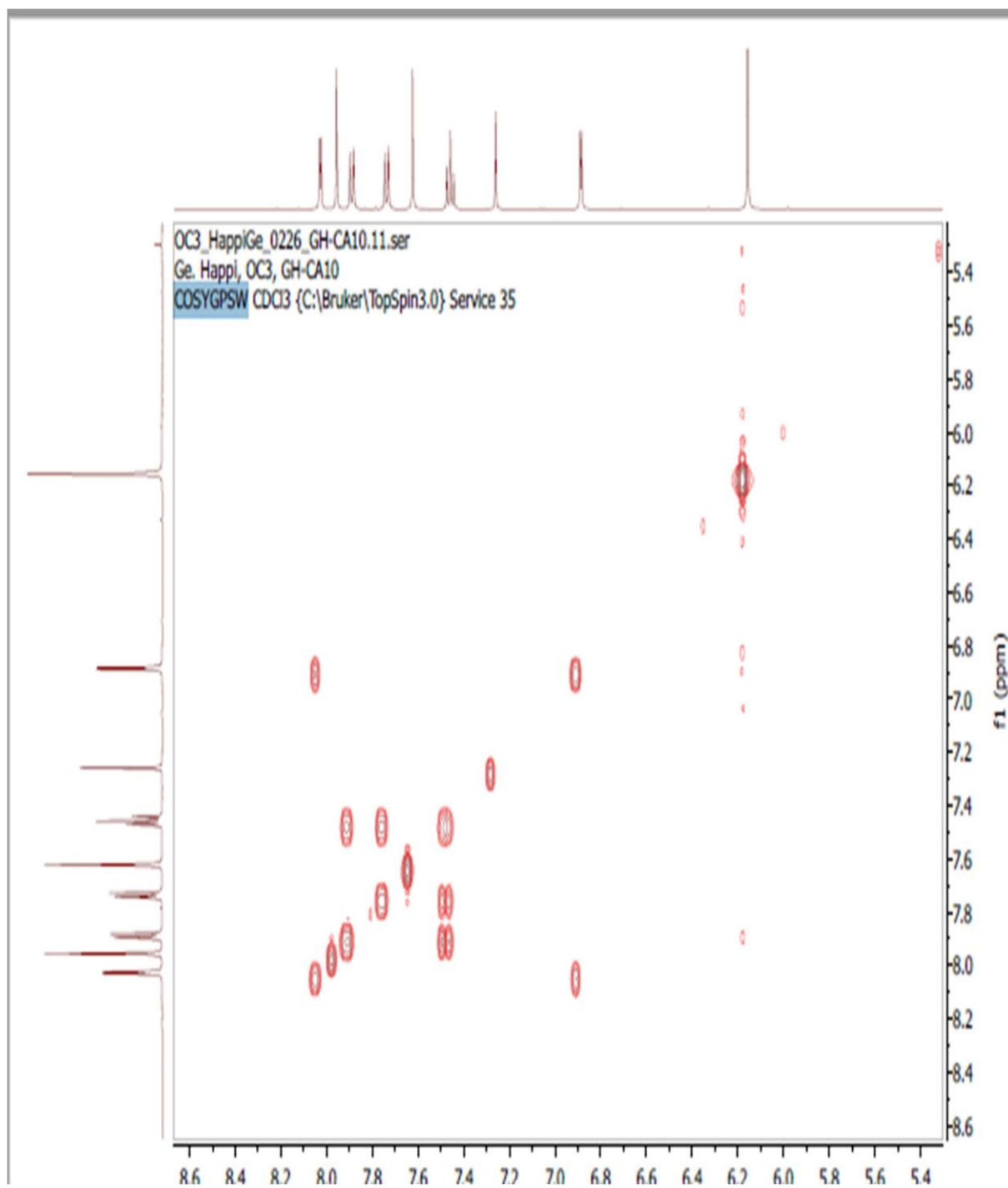
#### Annex 2.1A. $^1\text{H}$ NMR Spectrum of Hippadine ( $\text{C}_{16}\text{H}_9\text{NO}_3$ )(Ca10)



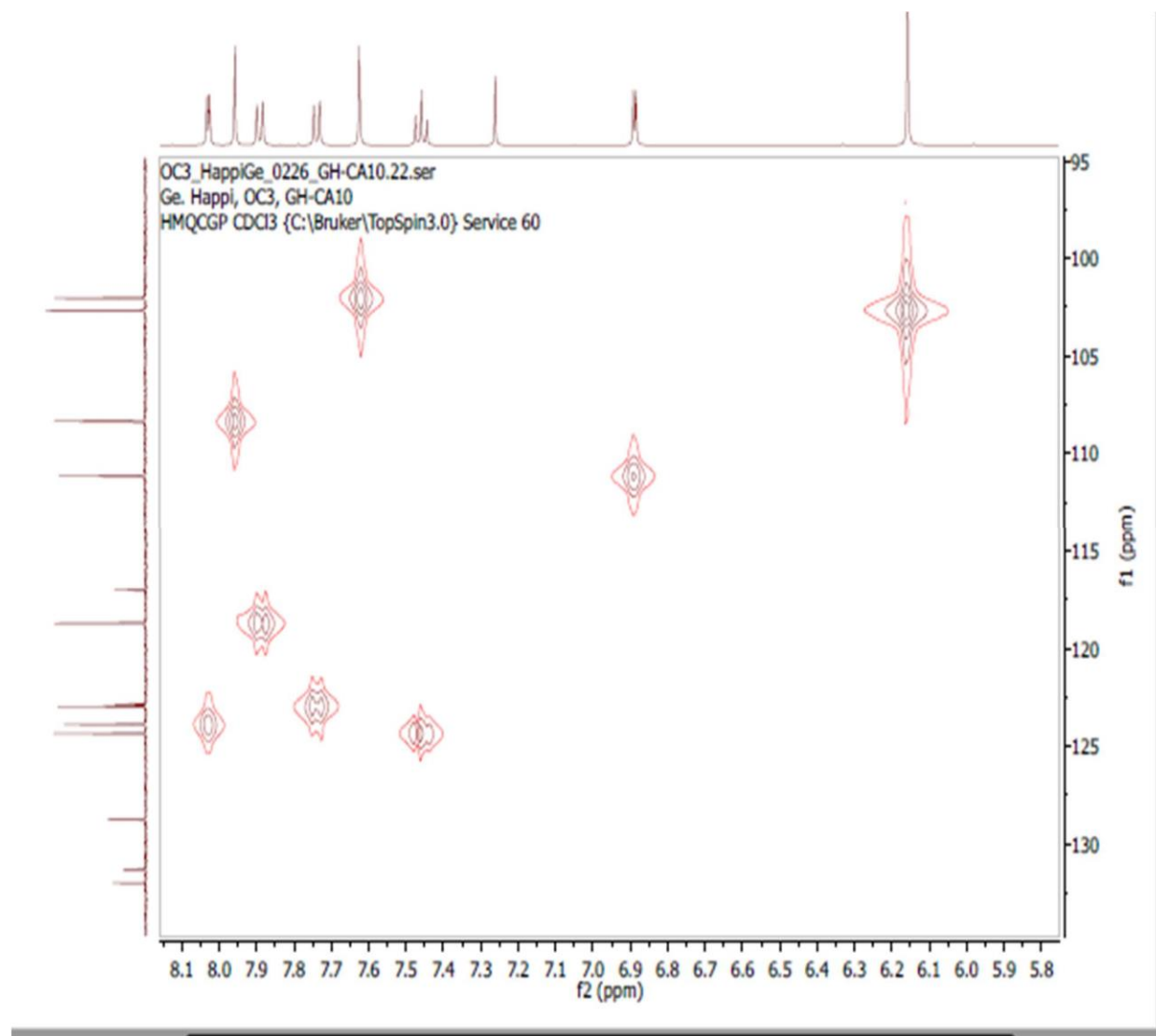
Annex 2.1B.  $^{13}\text{C}$ NMR Spectra of Hippadine ( $\text{C}_{16}\text{H}_9\text{NO}_3$ )(Ca10)



Annex 2.1C. Correlation Spectroscopy (COSY) of Hippadine (C<sub>16</sub>H<sub>9</sub>NO<sub>3</sub>)(CA10)

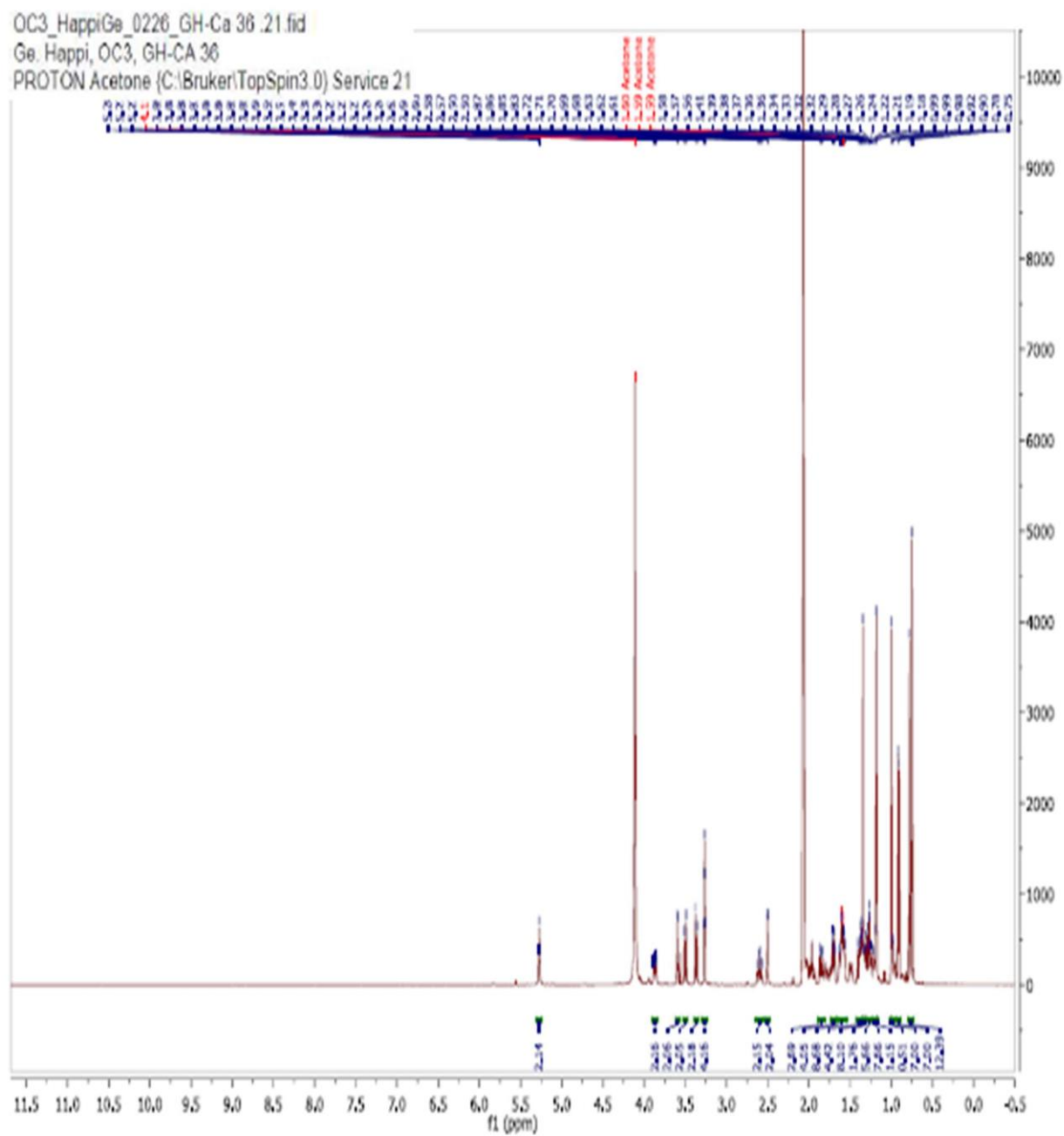


Annex 2.1D. HMBC Spectra Revealed A Correlation NMR Spectrum of Hippadine  
(C<sub>16</sub>H<sub>9</sub>NO<sub>3</sub>)(CA10)



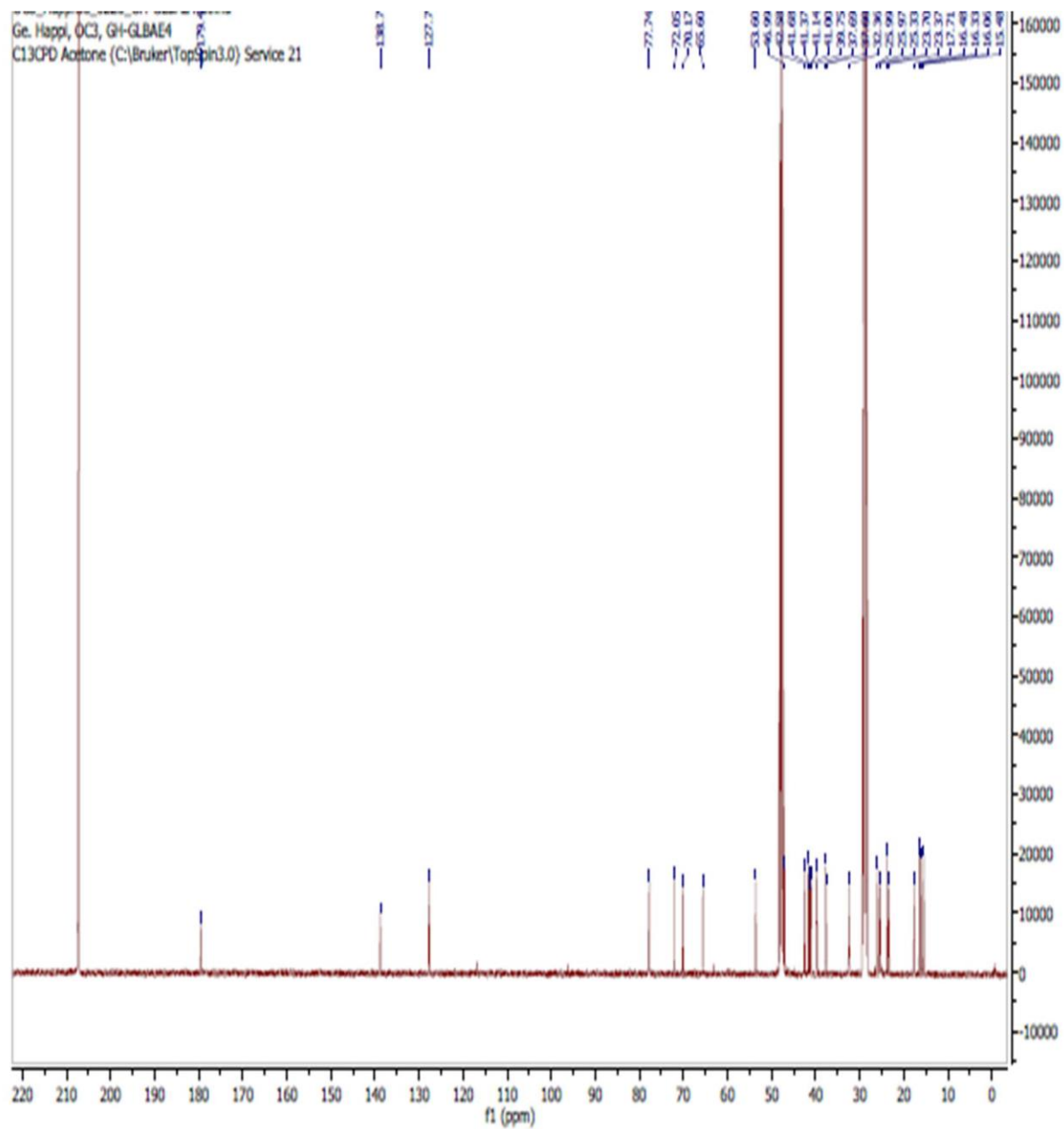
## Annex 2.2. NMR Spectras of Ca 36 Compound

### Annex 2.2A. $^1\text{H}$ NMR Spectrum of 2,3,19,23-tetrahydroxy-urs-12-en-28-oic acid (Myrianthic acid)(Ca 36)

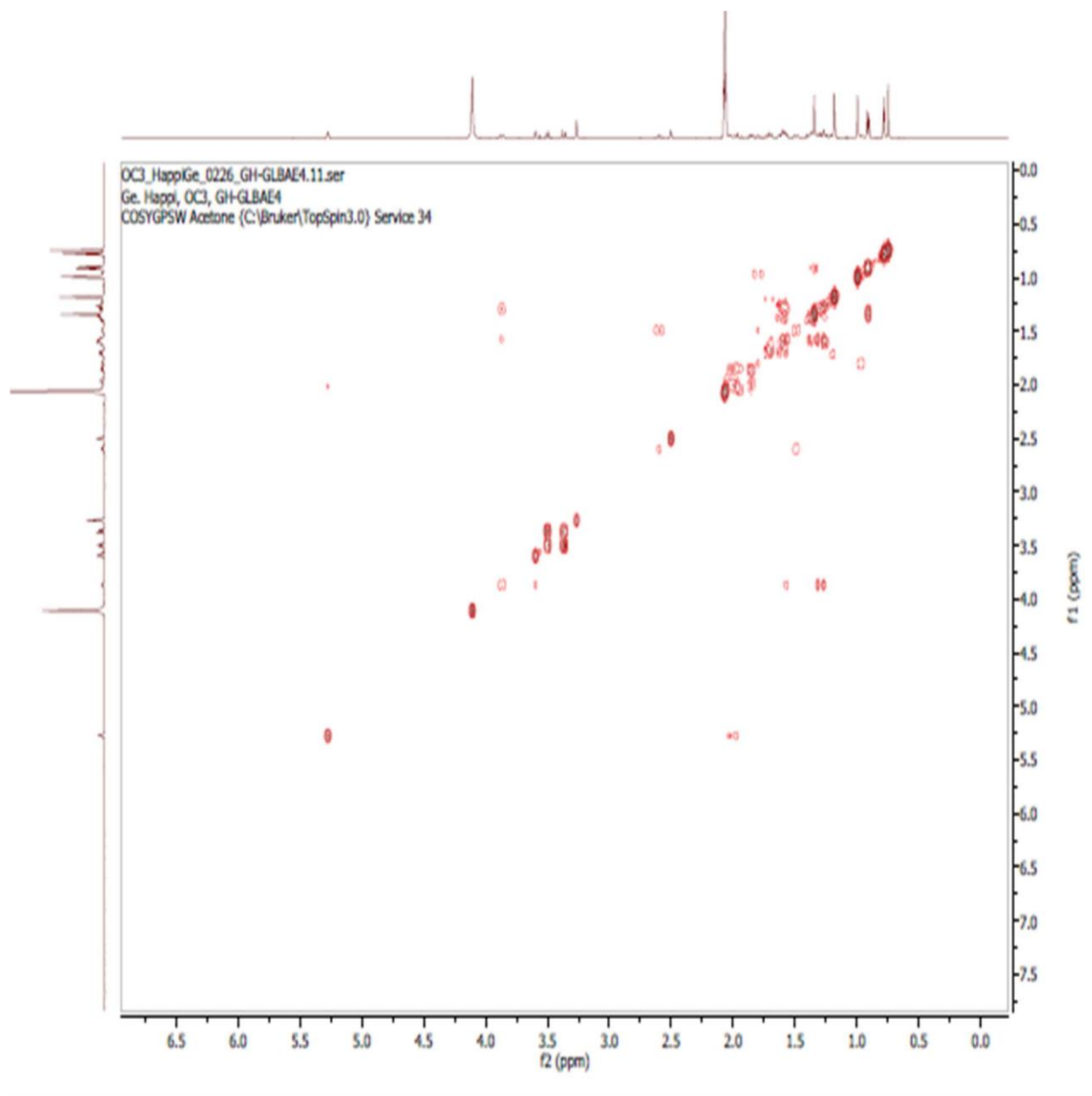




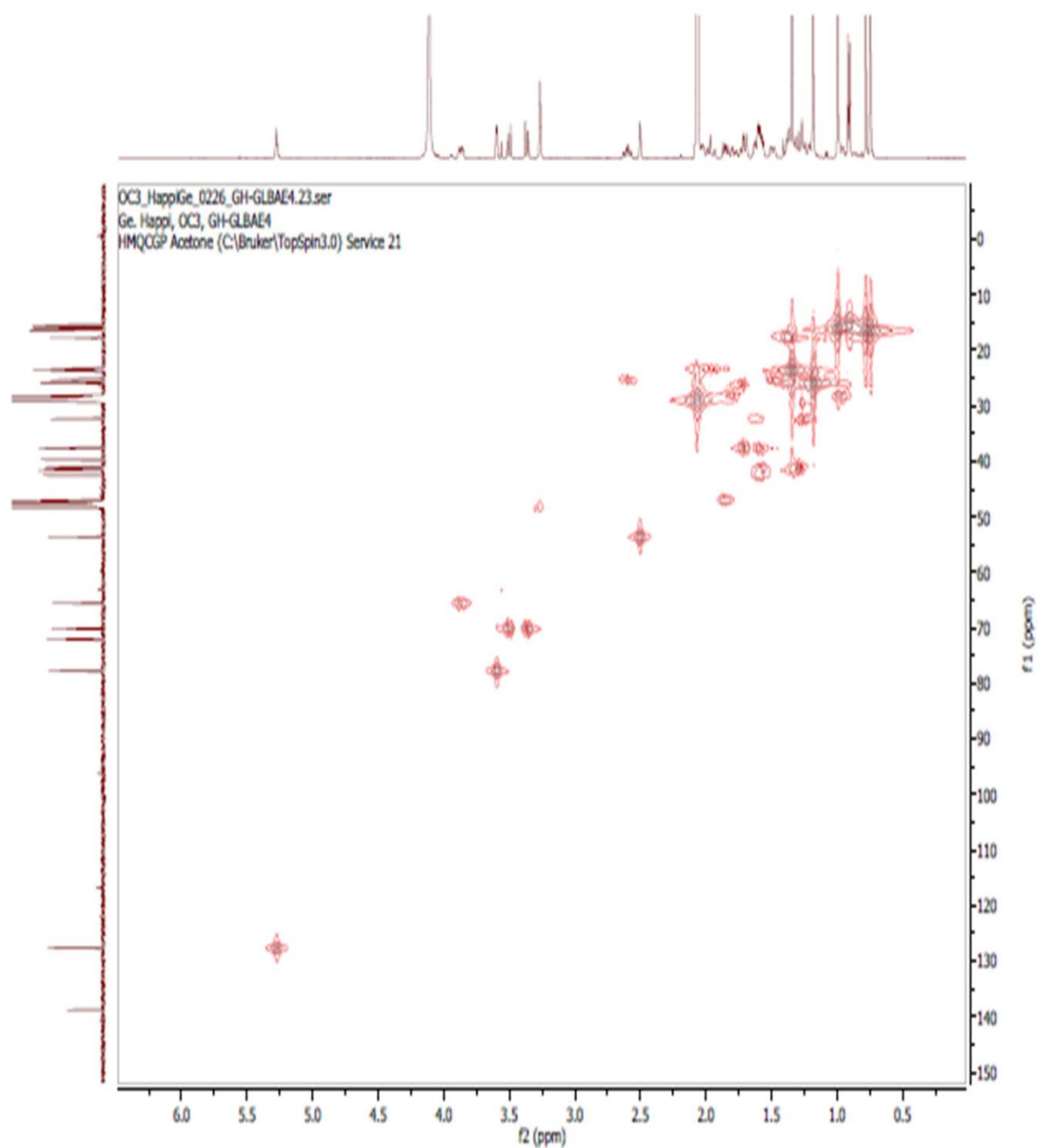
Annex 2.2B.  $^{13}\text{C}$  NMR Spectrum of 2,3,19,23-tetrahydroxy-urs-12-en-28-oic acid (Myrianthic acid)(Ca 36)



Annex 2.2C. Correlation Spectroscopy (COSY) of 2,3,19,23-tetrahydroxy-urs-12-en-28-oic acid (Myrianthic acid)(Ca 36)

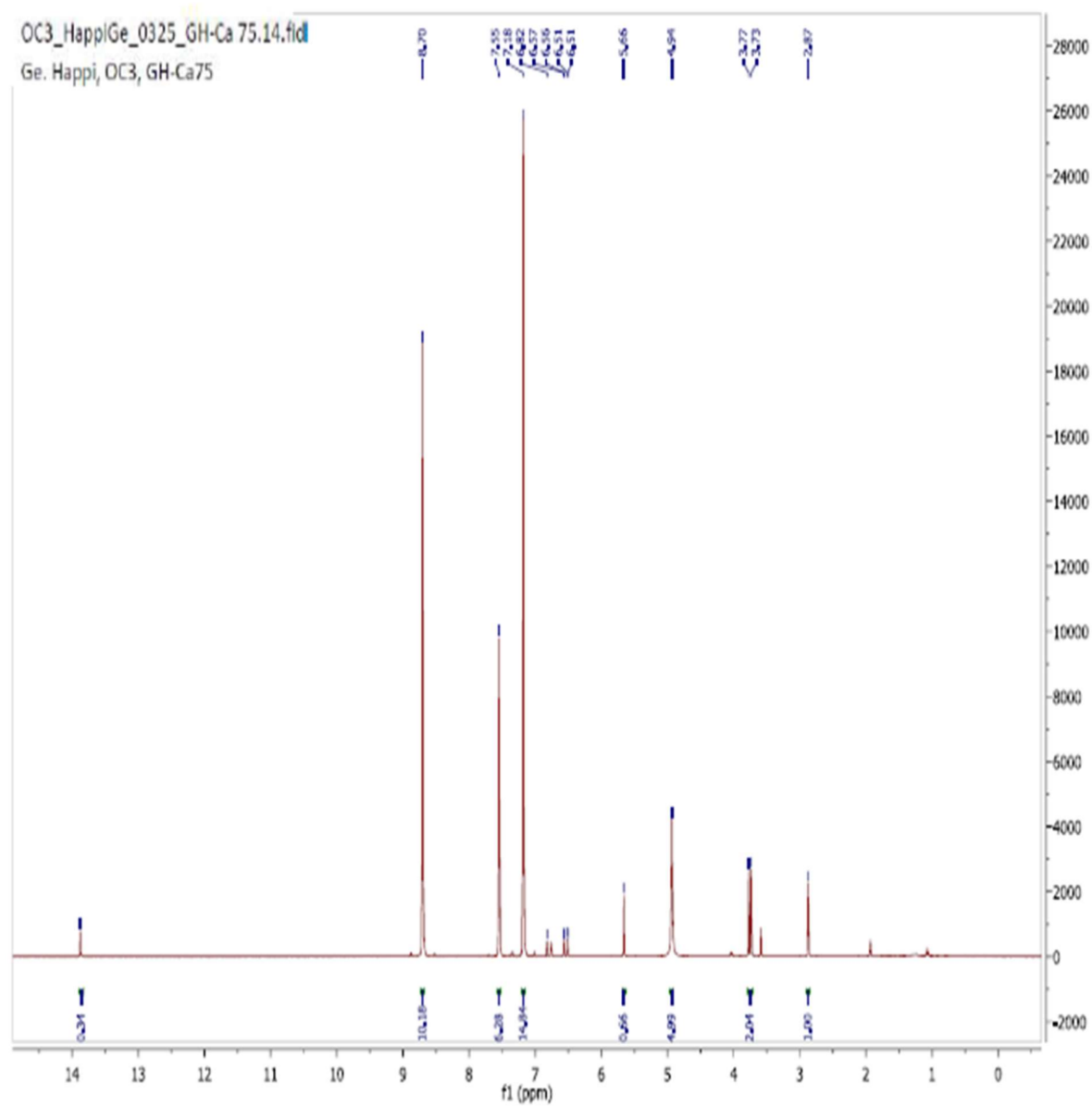


Annex2.2D. HMBC spectra revealed a correlation OF 2,3,19,23-tetrahydroxy-urs-12-en-28-oic acid (Myrianthic acid)(Ca 36)

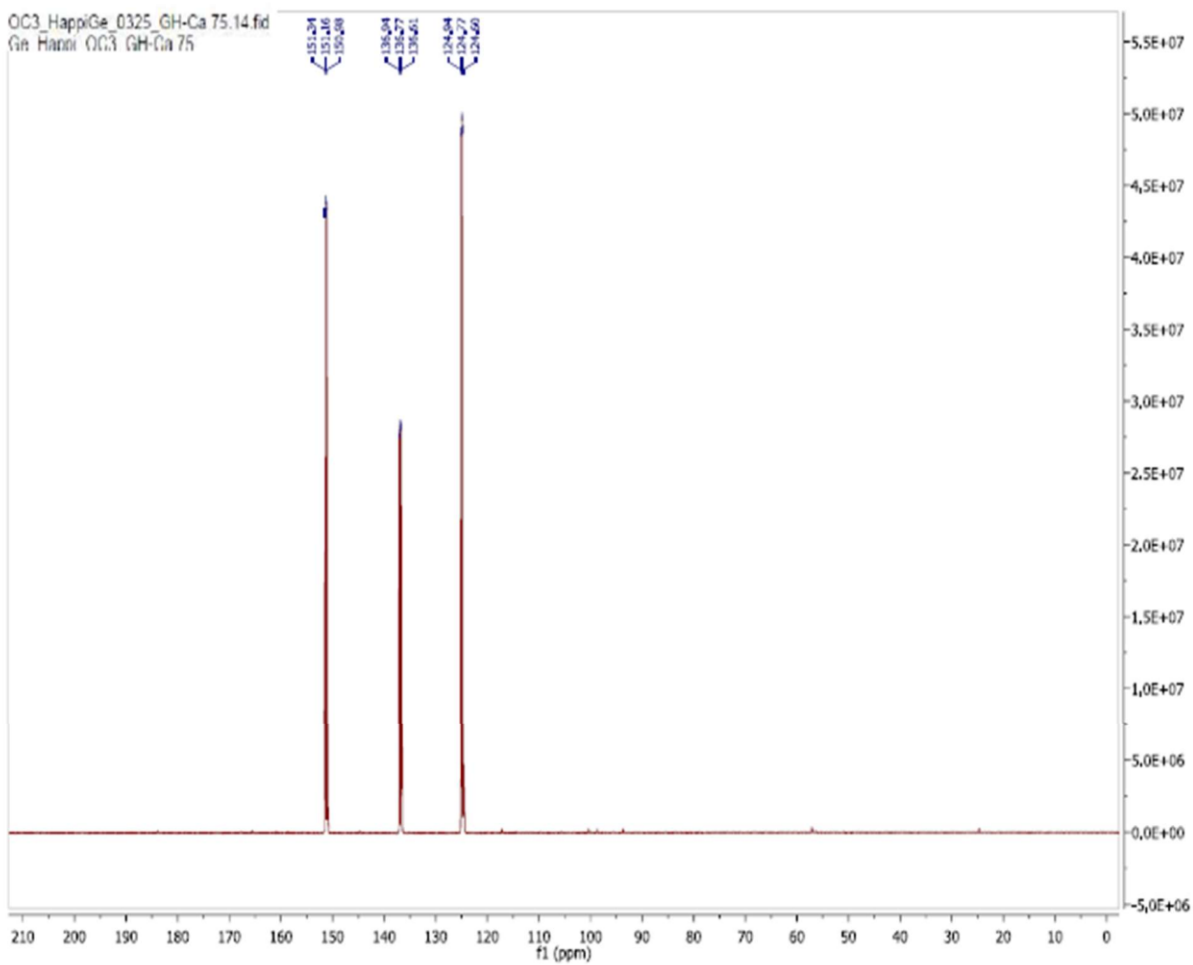


## Annex 2.3. NMR Spectras of Ca 75 Compound

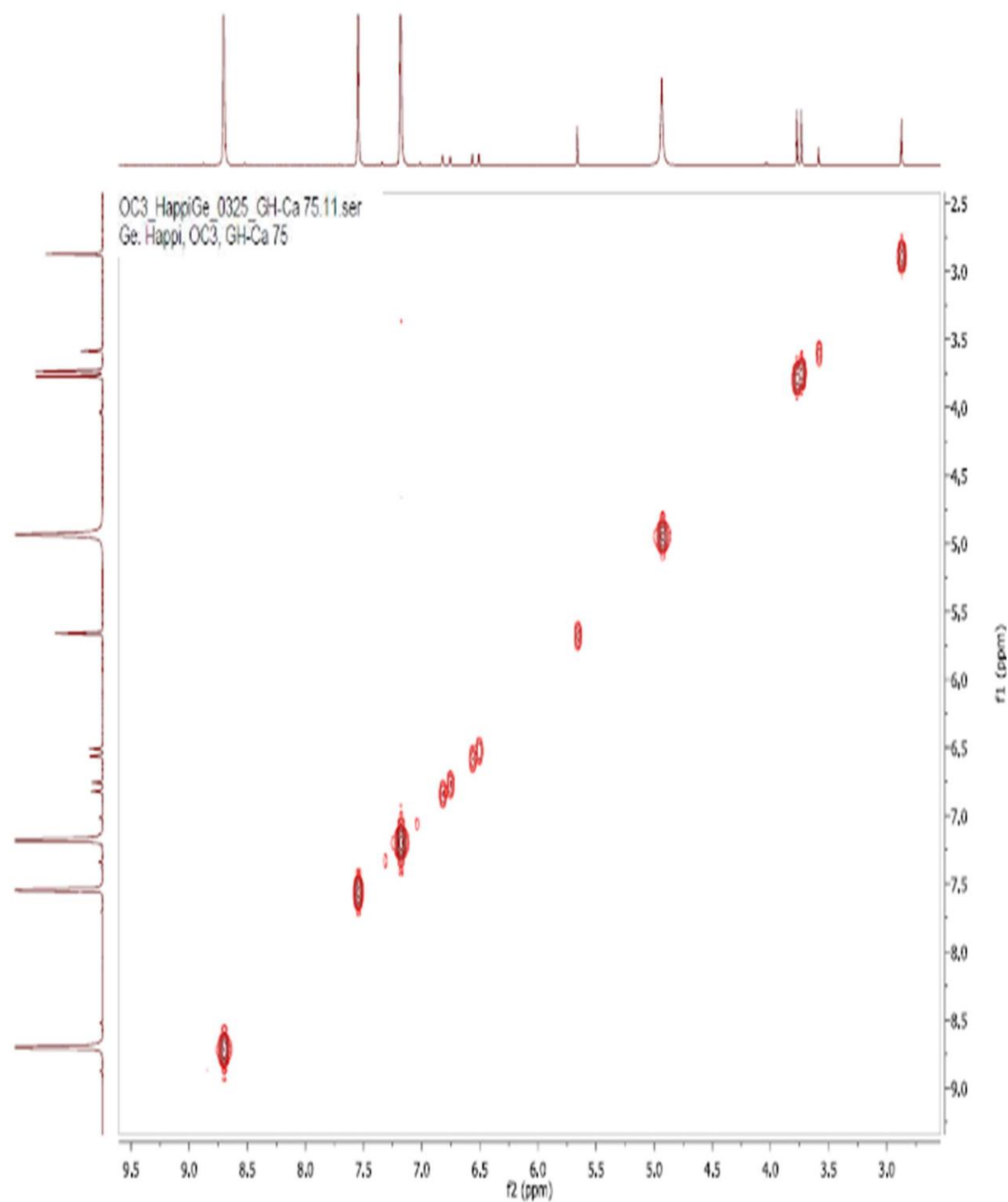
Annex 2.2A.  $^1\text{H}$  NMR Spectrum of 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone) (Ca 75)



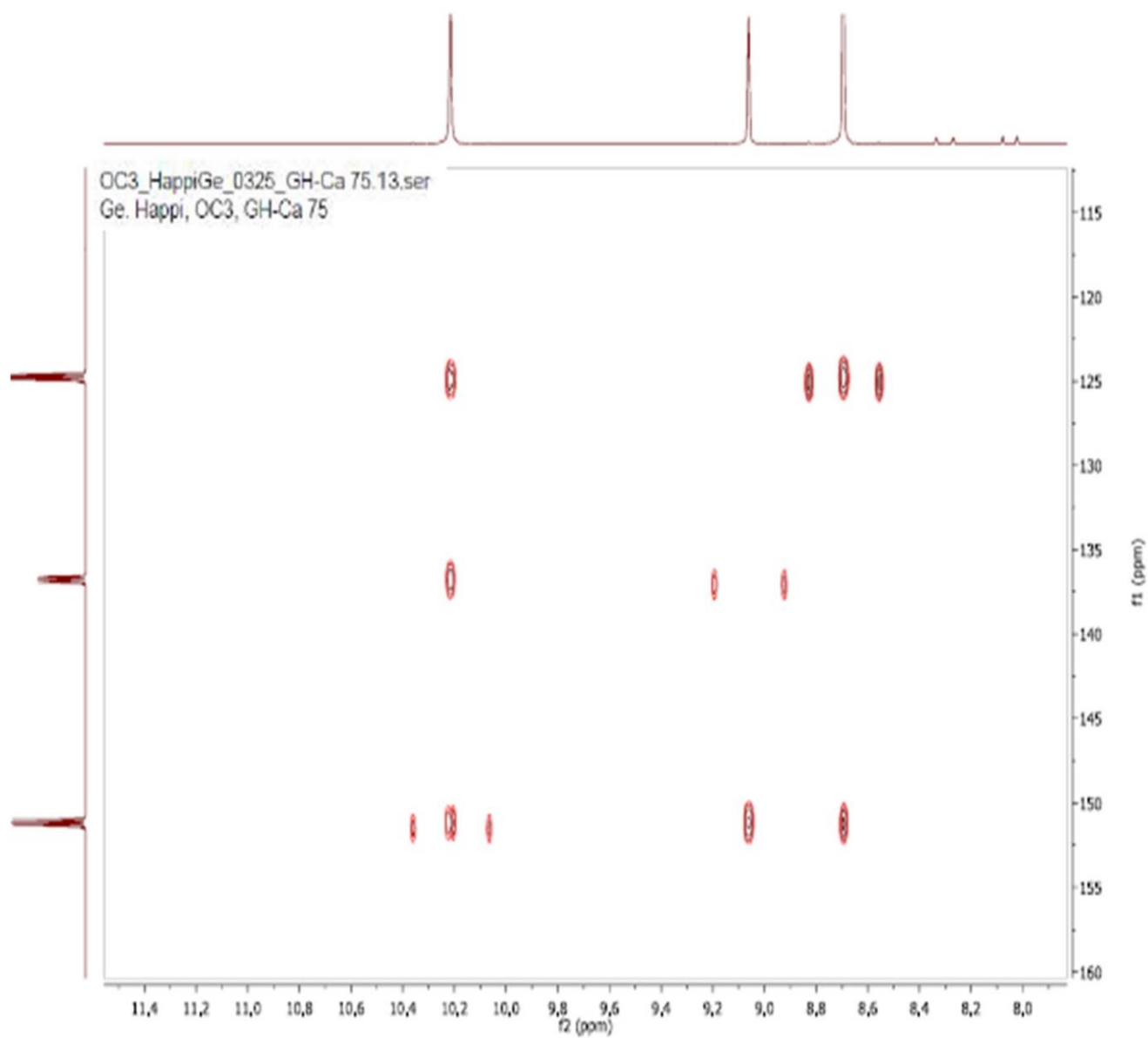
Annex 2.2B.  $^{13}\text{C}$  NMR Spectrum of 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone) (Ca 75)



Annex 2.2C. Correlation Spectroscopy (COSY) <sup>of</sup> 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (Lichexanthone) (Ca 75)



Annex 2.2D. The Heteronuclear Multiple Bond Correlation (HMBC) OF 1-Hydroxy-3,6-Dimethoxy-8-Methyl-9H-Xanthen-9-one (LICHEXANTHONE) (Ca 75)



### Annex III: Different Certificates

#### Yaoundé University Six Monthes Research Stay Training Testimony Certificate

 **Universität Bielefeld**

**Yaoundé-Bielefeld Graduate School**  
**YaBiNaPA**

Natural Products with Antiparasitic and Antibacterial Activity (YaBiNaPA)

**Prof Dr. LENTA NDJAKOU Bruno**  
Laboratory of Therapeutic Chemistry and Pharmacognosy  
Department of Chemistry/Ens Yaoundé  
University of Yaoundé I, POBox 47, Yaoundé Cameroon  
Email: lentabruno@yahoo.fr Tel. +237 675097561  
Coordinator of the YaBiNaPA Project

Yaoundé the

To  
**Mr SILESHI DUBALE DINSSA**  
POBox 318, Mettu University, Ethiopia  
Tel. +251917806080  
E-mail: sileshi.dubale@gmail.com

**Confirmation of Participation on a Research Internship as Part of the DAAD  
Funded Bilateral Graduate School YaBiNaPA (Yaoundé/Bielefeld)**

As part of the **DAAD funded Bilateral Graduate School YaBiNaPA**,

Mr SILESHI DUBALE DINSSA Was a guest in my research group at the University of  
Yaoundé I in the time from June 1<sup>st</sup> to November 30<sup>th</sup>, 2018.

In this project, Mr SILESHI investigated natural products from ethnomedicinally used  
plants from Ethiopia. He extracted the plant materials and screened the extracts for antimicrobial  
and antiparasite activity in vitro. Some compounds were isolated using usual chromatographic  
methods via normal phase, reverse phase and all this coupled to LC-MS data. Having recorded the  
LC-MS of the isolated compounds, pure samples were submitted for NMR analysis and were tested  
for antimicrobial activity.

During this period of internship, he successfully attended training on basic anti-microbial  
activity test technique, Good laboratory & good manufacturing practice, Principle of clinical trial,  
Natural product anti-cancer activity studies and basic principles of LC-MS and NMR analysis  
methods.

He impressed us with his politeness and collaboration. He plans his manipulations very well  
and possesses the intellectual ability required for an innovative research. He is hardworking, able  
to work under minimum supervision and relates well with others.

While thanking him for having chosen to do some of his thesis work at the Bilateral  
Graduate School YaBiNaPA, we wish him a rich and long-lasting career in Research

**The coordinator of YaBiNaPA Project**

  
  
**Prof. Dr. Lenta Ndjakou Bruno**  
Natural Products Chemi

**DAAD**

YaBiNaPA is supported by the DAAD with funds from the Federal Ministry for  
Economic Cooperation and Development (GIZ)

 **Federal Ministry  
for Economic Cooperation  
and Development**

Scanned By Camera Scanner



## Grant Writing Training Certificate



## Certificates of Different Conference Participation





**WOLLEGA UNIVERSITY**

ICON OF QUALITY AND RELEVANCE



RESEARCH, COMMUNITY ENGAGEMENT & INDUSTRY LINKAGE  
V/PRESIDENT OFFICE

# Certificate of Appreciation

THIS CERTIFICATE IS AWARDED TO

Mr./Ms./ Mrs./ Dr. SOLESHI DUBALE

For actively working as **PAPER PRESENTER** on the  
National Research Conference with Theme  
**"RESEARCH, TECHNOLOGY AND INNOVATION FOR  
RESILIENT AND DEVELOPMENT IN CHANGING ENVIRONMENT"**  
organized by **WOLLEGA UNIVERSITY** on 20 and 21 March 2022 GC  
at Main Campus, Nekemte.

**DIRIBA DIBA (PhD)**

RESEARCH, COMMUNITY  
ENGAGEMENT & INDUSTRY LINKAGE  
V/PRESIDENT



**HASAN YUSUF (PhD)**

UNIVERSITY PRESIDENT



## CERTIFICATE OF PARTICIPATION

THIS CERTIFICATE IS PRESENTED TO

**Mr. Sileshi Dubale**

who has presented his/her research paper entitled "Treatment of infections using Ethnomedicine and indegnous knowldges in South West Ethiopia" at the 6<sup>th</sup> Annual National Research Conference of Mettu University with the grand theme "*Blending Research and Indigenous knowledge to ensure Socio-Economic Transformation of the Nation*" which was held from May 7-8, 2022 at Mettu University, Ethiopia. Thus, we are pleased to give you this certificate of recognition to express our highest gratitude.

A blue ink signature of Tadele Mamo, consisting of stylized, overlapping loops and lines.

Tadele Mamo (PhD)  
RPDD Directorate Director



A blue ink signature of Bedilu Teka, consisting of stylized, overlapping loops and lines.

Bedilu Teka (PhD)  
Res/CS/Col/Vice President

## Annex V: Plagiarism checker Certificate

I, the supervisor of the PhD dissertation entitled **Ethnomedicinal Plants for Infectious Diseases in Ethiopia: Traditional Use, Antimicrobial & Antibiotic Modulating Effects, Phytochemistry, and Regulatory Perspectives** certify that it has been tested for plagiarism (<15%).

Name: **Prof. Dr. Sultan Suleman Wega**

Signature: \_\_\_\_\_



Date: **17<sup>th</sup> April 2024**