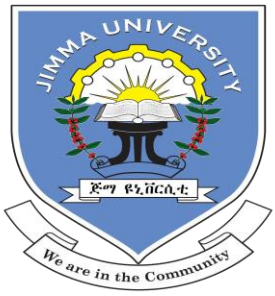

SCHOOL OF POSTGRADUATE STUDIES

COLLEGE OF NATURAL SCIENCE

DEPARTMENT OF BIOLOGY

ANTIMICROBIAL AND FOOD PRESERVATIVE ACTIVITIES OF SELECTED MEDICINAL PLANTS IN SEKA CHOKORSA DISTRICT, JIMMA ZONE, SOUTHWEST ETHIOPIA

BY

CHALA DANDESSA

OCTOBER, 2019

JIMMA, ETHIOPIA

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A THESIS SUBMITTED TO DEPARTMENT OF BIOLOGY, JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED MICROBIOLOGY

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College of Natural Sciences

School of Graduate Studies

Antimicrobial and Food Preservative Activities of Selected Medicinal Plants In Seka Chokorsa District, Jimma Zone, Southwest Ethiopia

BY

Chala Dandessa Debela

A Thesis presented to School of Graduate Studies, Jimma University, in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Applied Microbiology)

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Abstract

Antimicrobial activity is the activity of either crude of the plant or medicine that can stop or eliminate the growth of microbes. Natural food preservation method is application of plant preservatives to prevent food spoilage and growth of food borne pathogens in food and foods products. The main purpose of the study was to assess antimicrobial and food preservative activity of selected medicinal plant in Seka Chekorsa, one of the districts in Jimma Zone, Oromia Region of Ethiopia. Commonly used medicinal plants by most of the practitioners were selected based on frequency from the medicinal plant practitioners. Based on this, Croton macrostachyus, Calpurnia aurea and Vernonia myriantha were collected, well dried, grinded by using coffee grind machine and then soaked in chloroform, acetone and methanol. After filtering using filter paper the solvents were removed using rotary evaporator. By re-dissolving the crude in DMSO antimicrobial activity of all crudes were conducted. Testing strains for antimicrobial activity (Staphylococcus aureus, Bacillus cereus, Eschercheria coli and Salmonella thyphurium) were isolated, while available strain of Candida albicans was used. After evaluating the antimicrobial activities of the plant crude extracts chloroform extracted Croton macrostachyus showed antimicrobial activity on S. aureus which is 23.33 ± 3.30 mm, while methanol extracted Croton macrostachyus showed good antimicrobial activity on C. albicans which is 22.33 ± 1.70 mm. long. Actone extracted croton macrostachyus also showed antimicrobial activity on S. aureus by 17.67 ± 2.05 mm, on C. albicans by 19.67 ± 0.94 mm, methanol extracted croton macrostachyus showed by 12.67 ± 2.05 mm, on C. albicans 22.00 ± 0.82 mm. Phytochemical screening of the crude showed that, alkaloids, flavonoids, saponin, phenols, tannins, terpenes and steroids were detected in the crude both crude of Croton macrostachyus and Calpurea aurea. MIC of those crude were determined by serially diluting at different folds. The MIC of C. macrostachyyus on B. cereus was 0.267 g/ml, 0.235 g/ml on S. aureus, 0.182 g/ml on C. albicans. In food preservative activity the mean log of 10 of the cfu in *Zingiber officinale 6.08* and 2.49, in *Moringa oliefera* 3.54 and 2.62, in *Allium sativum* 3.60 and 2.72 control and experimental respectively. *Generally, phytochemicals in Croton macrostachyus, Calpurnia aurea and Vernonia myriantha have good antimicrobial and while those in Zingiber officinale, Moringa oliefera and Allium sativum good food preservative activities. Therefore, scholars should work on the development study on the issue.*

Keywords: Croton macrostachyus, Food preservative activity, Moringa oliefera, Seka Chekorsa,

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Abbreviations

AMR:	Antimicrobial Resistance
ATP:	Adenosine Triphosphate
DNA:	Deoxyribonucleic Acid
EO:	Essential Oils
DMSO:	Dimethyl Sulphoxide
$FeCl_3$:	Iron Trichloride
HCl:	Hydrochloric Acid
H_2SO_4:	Sulphuric Acid
MBC:	Minimal Bactericidal Concentrations
MIC:	Minimum Inhibitory Concentration
SPSS:	Software Package for Social Science
WHO:	World Health Organization

1. Introduction

Antimicrobial activity is ability of either medicine or plant crudes that can inhibit growth of microorganisms(Sahraei *et al*., 2014). Herbs and spices containing essential oils (EOs) have demonstrated activity against pathogens, such as *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus*, in food systems *(Ahmed et al.*, 2013; Tajkarimi *et al*., 2010).

Food preservation may be defined as the process of treating and handling food in such a way as to stop, control, or greatly slow down spoilage and, of course, to minimize the possibility of foodborne illness while maintaining the optimum nutritional value, texture, and flavor(Sancho-Madriz, 2003a). Food preservation consists of the application of science-based knowledge through a variety of available technologies and procedures, to prevent deterioration and spoilage of food products and extend their shelf-life, while assuring consumers a product free of pathogenic microorganisms. Shelf-life may be defined as the time it takes a product to decline to an unacceptable level. Deterioration of foods will result in loss of quality attributes, including flavor, texture, color, and other sensory properties(Sancho-Madriz, 2003b).

Microbial food safety is a constant, global problem affecting the health of consumers*(Antolak et al.*, 2017). In other case the health and economic burden associated with antimicrobial resistance to antibiotics currently being developed by microbes on a global scale are alarming. Natural food preservation method is application of naturally produced antimicrobial compounds that are obtained from plants, animals and microbes to prevent food spoilages microorganism, proliferation and growth of food borne pathogens in food and foods products(Amenu, 2016). Antimicrobial compounds derived from plants products are considered to be an excellent source of natural food preservatives(Amenu, 2016).

Although there were many scholars who conducted the study to document locally available medicinal plants empirical or local knowledge of traditional healers on commonly used medicinal plants in the study area, there was no reported study on antimicrobial and food preservative activities of the extracts of medicinal plants from the study area (Abera, 2003).

Because of synthetic chemical preservation raised many safety issues, alternative sources of natural, bioactive, and antimicrobial compounds are needed. Plant extracts are a potentially useful source of antimicrobial compounds(Antolak *et al.*, 2017; Sahraei et al., 2014). Phytochemicals in plants are broadly grouped into phenolic compounds, terpenoids and essential oils, alkaloids, lectins and polypeptides(Amenu, 2016). The main purpose of current study was to evaluate antimicrobial and food preservative activity of selected medicinal plant currently used in Seka Chekorsa district of Jimma Zone.

1.1. Statement of the Problem

Antimicrobial resistance (AMR) is currently one of the major threats facing mankind. The health and economic burden associated with AMR on a global scale are dreadful. Development of new, effective and safe antimicrobials is one of the ways by which AMR burden can be reduced. Medicinal plants are potential sources of new antimicrobial molecules. There is continuous and urgent need to discover new antimicrobial compound with diverse chemical structures and novel mechanism of action. Food products are subjected to contamination by microorganisms causing undesirable changes including food borne illness. An internationally acceptable standard in food quality emphasized that food (processed or raw) should be wholesome and free of contaminants. To prevent spoilage of food several physical and chemical preservation techniques are commonly employed. Nowadays, there is an increasing consumer awareness concerning the use of processed food having no chemical preservatives. The trend to be supported today is the use of natural additives or preservatives instead of any chemical additives. Medicinal plants are potential source of novel compounds of antimicrobial and food preservative activities.

The basic research questions were:

- Which medicinal plant is most commonly used in the study area?
- Are the medicinal plants effective for antimicrobial and food preservative activities?
- What is the minimal inhibitory concentration (MIC) of extracts that display antimicrobial activity?

1.2. Objectives of the Study

1.2.1. General Objective

The general objective of the study was:

- To assess antimicrobial and food preservative activities of selected medicinal plants, in Seka Chekorsa District, Southwest Ethiopia

1.2.2. Specific Objectives

The specific objectives of the study are:

- To assess the traditional medicinal plants commonly used community of the study area
- To extract and screen phytochemicals of selected medicinal plants
- To isolate and characterize the food borne pathogens
- To determine antimicrobial activities and minimum inhibitory concentration of the extracts of different medicinal plants
- To evaluate food preservative activity of selected medicinal plants

1.3. Significance of the Study

Medicinal plants are gaining much interest recently because their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. Current study aimed to evaluate antimicrobial and food preservative. Identification of these traditional medicinal plants helps the local communities to use it as an alternative means of curing the diseases by the modern drugs. It will also contribute to aware the communities to the hygienic preparation and usage of the traditional medicinal plants. In Ethiopia, ministry of health focused on the traditional medicinal plants to achieve its goal. Therefore, the study will also have importance for the country. Scientifically evaluating the antimicrobial and food preservative activity of the extracts of selected medicinal plant will ensure the community to use the plants without any disbelief.

Generally, this study has great importance in; improving the poor documentation of medicinal plants in Seka Chekorsa District, initiate the researchers to study more and evaluate antimicrobial and food preservative activities of different medicinal plants, to enrich the existing literature on the use of medicinal plants and to be used as base line data for further study.

2. Literature Review

2.1. Medicinal Plants

Medicinal plants can be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans. These plants are either "wild plant species" those growing spontaneously in self-maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or the contrasting "Domesticated plants species" those that have arisen through human actions such as selection or breeding and depend on management for their existence(Oladeji, 2016). Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention(Azwanida, 2015).

2.2. Literatures on Nutraceutical Plants

The characteristics of herbs are conditioned by their chemical composition, e.g. its aroma and flavour depend on the volatile oils such as monoterpenes. Among other things, these features made them recognized as a food, food additives and in the manufacturing of cosmetics and pharmaceuticals. What is more, global awareness of health and environmental issues, especially in the developed countries, caused increased demands for medical herbs and spices as well as food products containing these plant materials. Increasingly common are new herbs or mixtures of herbs designed to improve the health of consumers(Antolak *et al.*, 2017). According to Sanchez *et al. (2010),* the use of natural compounds from plants can provide an alternative approach against foodborne pathogens. Changes in membrane integrity, membrane potential, internal pH (pH_{in}), and ATP synthesis were measured in *Vibrio cholerae* cells after exposure to extracts of edible and medicinal plants. Extracts from these plants were able to disrupt the cell membranes of *V. cholerae* cells, causing increased membrane permeability, a clear decrease in cytoplasmic pH, cell membrane hyperpolarization, and a decrease in cellular ATP concentration in all strains tested. These four plant extracts could be studied as future alternatives to control *V. cholerae* contamination in foods and the diseases associated with this microorganism*(Sánchez et.*

al, 2010). It has long been shown that phytochemicals protect plants against viruses, bacteria, fungi and herbivores, but only relatively recently we have learnt that they are also critical in protecting humans against diseases. A significant amount of medicinal plants is consumed by humans. As food-related products, they additionally improve human health and general well-being(Antolak *et al.*, 2017).

Allium sativum is a plant which is native to Central Asia. It is used commonly for its pungent odour and taste. The garlic bulb is the most commonly used part of the plant. It is supposed to express antimicrobial activities and thus its potential needs to be explored. Garlic and cinnamon used for food preservation because of their bacteriocin based strategies. Since it is easily available worldwide it should be consumed in order to prevent any microbial infection*(Verma et al.*, 2012).

2.3. Extraction of Medicinal Plants

Extraction is the separation of medicinally active portions of plant using appropriate solvents following standard procedures. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing (Azwanida, 2015).

A typical extraction process may involve following steps: (a). Collection and authentication of plant material & drying, (b) Size reduction, (c) Extraction, (d) Filtration, (e) Concentration, (f) Drying & reconstitution. Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material: solvent ratio etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction (Gupta *et al.*, 2012).

Different types of solvents can be used while extraction of plant extracts including methanol, propanol and acetone. According to Verma *et. al*, 2012, four solvents were used to prepare plant extracts namely Methanol (85%), Propanol (85%), Acetone (85%) & Water (Verma *et al*., 2012).

2.4. Antimicrobial Activities of Medicinal Plant Extracts

Five plant species were investigated to evaluate their antibacterial activity against food poisoning bacteria including two strains of Gram positive bacteria (B. cereus & S. aureus) and three strains of Gram negative bacteria (E. coli, S. typhi & P. aeruginosa) using disc diffusion method(Mostafa *et al*., 2018). It has been documented that M. piperita extracts and EOs are characterized by broad spectrum of antibacterial activities against gram-positive and gram-negative pathogens as well as antifungal activities against yeasts and moulds. In the study of Singh *et al*., it was found that gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus pyogenes* are more sensitive to essential oil compared to *Escherichia coli*. Authors also established that the growth inhibition was compared with gentamycin. Mint oil also had bactericidal effect against Staphylococcus mutans, Salmonella typhimurium, Pseudomonas aeruginosa and *Shigella spp*(Antolak & Kregiel, 2017). According to Sanchez et al, preliminary screen of methanolic, ethanolic, and aqueous extracts of medicinal and edible plants was performed. Minimal bactericidal concentrations (MBCs) were measured for extracts showing high antimicrobial activity. Their results indicate that methanolic extracts of basil (*Ocimum basilicum* L.), nopal cactus (*Opuntia ficus-indica* var. Villanueva L.), sweet acacia (*Acacia farnesiana* L.), and white sagebrush (*Artemisia ludoviciana* Nutt.) are the most active against *V. cholera*, with MBCs ranging from 0.5 to 3.0 mg/ml. Using four fluorogenic techniques, they studied the membrane integrity of *V. cholerae* cells after exposure to these four extracts(Sánchez *et al*., 2010).

Antimicrobial activity of EOs extracted from Thymus vulgaris (thymol chemotype), Thymus zygis subsp. gracilis (thymol and two linalool chemotypes) and Thymus hyemalis L. (thymol, thymol/linalool and carvacrol chemotypes) was active against 10 pathogenic micro-organisms (Sabulal, George, Pradeep, & Dan, 2008). Generally, Gram-positive bacteria are more sensitive to saponin, with MIC (minimum inhibi- tory concentration) between 0.3 and 1.25 mg/ml, compared to 1.25–5 mg/ml for Gram-negatives(Tajkarimi *et al*., 2010).

Antimicrobial activity of spices with methanolic extract of Zingiber officinale (Ginger), Allium sativum (Garlic), Syzygium aromaticum (Clove), Cuminum cyminum (Cumin), Brassica juncea (Musturd), Emblica officinalis (Amla), Aloe vera and Crocus sativus(Saffron) has been evaluated against isolated food borne pathogens E.coli, Bacillus subtilis, Pseudomonas

florescens, Serratia marscens, Citrobacter frendii, Kleibsiella pneumonia, Staphylococcus aureus and Proteus vulgaris. They were tested by agar dilution method for determining the minimum inhibitory concentration (MIC) of spices extracts. Syzygium aromaticum (Clove) and Cuminum cyminum (Cumin) extract showed excellent antimicrobial activity against all the test organisms. Syzygium aromaticum showed the highest 19 mm antimicrobial zone against all except Serratia marscens and Proteus vulgaris. Clove extract was the most inhibitor followed by Cumin, whereas extracts of ginger, garlic mustard, amla aloe vera and saunf showed weak antibacterial activities against the tested strains. The most sensitive strain to spices extracts was Citrobacter frendii and the most resistant strain was Proteus vulgaris(Sethi *et al.*, 2013).

2.5. Phytochemicals of Extract of Medicinal Plants

Phenolic compounds are widely distributed, and an important group of compounds occurs in plants. Polyphenol family contains about 8000 structurally different compounds, commonly found in fruits, vegetables, seeds, flowers and leaves. They are generally categorized as phenolic acids and derivatives, flavonoids, tannins, stilbenes, lignans, quinones and others based on the number of phenolic rings and of the structural elements that link these rings(Antolak *et al.*, 2017). The antimicrobial activity of plants is as a result of certain compounds regarded as active compounds. These substances are naturally produced in plants as defense mechanisms against pathogenic microorganisms and insect pests. Plant phytochemicals are classified broadly as Terpenoids, Phenolics and Alkaloids(Fatoki *et al*., 2013).

A. Phenols

Phenols and their derivatives which possess oxygen molecules are secondary metabolites. They generally include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins(Fatoki *et al*., 2013). Phenols and phenolic acids are bioactive phytochemicals consisting of a single substituted phenolic ring. They contain varying number of hydroxyl groups and this determines the level of toxicity to microorganisms. Flavones, flavonoids and flavonols have the phenolic structure with one carbonyl group. Phenolics inhibit adhesion in some pathogens as well as disintegrating the outer membrane of bacteria leading to the cell becoming permeable hence affecting cell integrity. This disintegration is achieved by chelating divalent cations from the membrane(Fatoki *et al*., 2013).

B. Terpenes

Essential oils contain many substances including isoprene structure based substances called terpenes and terpenoids. Terpenes are known to disrupt membranes in microorganisms, alter their permeability and affect their ability to effectively carry out osmoregulation. Moreover, the ability to remove toxic substances through the microbial cell membrane is also greatly impaired. The 1, 8 cineole is a terpenoid with the ability to reduce growth, inhibit the spore production in fungi and germination of wide range of microbes. Since fungi absorb nutrients from their environment, they have been found to absorb terpenoids which lead to hyphae malformations, disorganization of cell wall, and leakage of cytoplasmatic material(Fatoki *et al.*, 2013).

C. Alkaloids

The diversity of alkaloids is an indication of their efficiency in antimicrobial activities of plant extracts. These compounds occur in varying concentrations in different plants and plant parts as well as having derivatives themselves that are all efficient against microbes. Some of these substances are lipohilic and hydrophobic in nature thereby altering the integrity of the cell wall and mitochondria while affecting the transport system and causing cell content leakages. They have also been found to be able to intercalate with DNA(Fatoki *et al.*, 2013).

2.6. Food Preservative Activities of Medicinal Plants

In addition to their flavoring effects, some spices and herbs have antimicrobial effects on plant and human pathogens. Food processing technologies such as chemical preservatives cannot eliminate food pathogens such as *Listeria monocytogenesis* or delay microbial spoilage totally(Tajkarimi et al., 2010). Preservative agents are required to ensure that manufactured foods remain safe and unspoiled. A number of Essential oils (EO) components have been identified as effective antibacterial, e.g. carvacrol, thymol, eugenol, cinnamaldehyde and cinnamic acid, having minimum inhibitory concentrations at higher dilutions in vitro(Ahmed et al., 2013). According to Ahmed *et al.*, 2013 potency of naturally occurring antimicrobial agents or extracts from plants, ranges of microbial susceptibility and factors influencing antimicrobial action and their anti-oxidative properties, aimed at food preservation, were reviewed*(Ahmed et al.*, 2013).

Spices and herbs can be used as an alternative preservative and pathogen-control method in food materials. Application of both extracts and EOs of plant-origin antimicrobials such as floral parts of *Nandina domestica* Thunb could be a potential alternative to synthetic preservatives(Tajkarimi *et al*., 2010). Plant-origin antimicrobials are present in a variety of plants, spices and herbs. Spices and herbs are used for both flavoring and preservation purposes. Spices and herbs, which were originally added for improving taste, can also naturally and safely improve shelf life of food products(Tajkarimi *et al*., 2010). Preservative agents are required to ensure that manufactured foods remain safe and unspoiled. Antimicrobial properties of essential oils reveal that Gram-positive bacteria are more vulnerable than Gram-negative bacteria. A number of EO components have been identified as effective antibacterial, e.g. carvacrol, thymol, eugenol, cinnamaldehyde and cinnamic acid, having minimum inhibitory concentrations at higher dilutions in vitro. Essential oils comprise a large number of components and it is likely that their mode of action involves several targets in the bacterial cell(Ahmed *et al*., 2013).

Food spoilage is a common problem faced all over the world. High temperatures, humidity, unhygienic preparation of food, lack of proper storage, etc. all contribute to food spoilage and growth of pathogenic bacteria that cause food illness. The chemical antibiotics have side effects on host and also destroy the natural micro flora of the body. In contrast the natural plant extracts

offer a very safe medical treatment with no side effects(Verma *et al.*, 2012). Application of dried plant extracts is a safe technique of preserving canned food. It will not have any kind of side effects but, it will increase the shelf life of the packed food. On the other hand food poisoning caused by food cooked in homes & not stored properly is very common because we often don't pay attention to it. Thus intake of plant extracts by people can prevent the ill effects of spoiled food. This is the easiest way to keep oneself fit in a natural way(Verma *et al*., 2012).

2.7. Common Foodborne Disease and Its Corresponding Pathogens

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption. Food borne sickness is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that infect food & we consume that spoiled food consciously or unconsciously. Food borne illness usually arises from improper handling, preparation, or food storage where hygienic approach is not followed. There is potential for a wide range of food products to become contaminated with microorganisms. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae. Of these, Salmonella and Escherichia coli are of particular concern. Other bacteria commonly responsible for food spoilage are Bacillus cereus, Staphylococcus aureus, etc. Apart from diseases caused by direct bacterial infection, some food borne illnesses are caused by exotoxins which are excreted by the cell as the bacterium grows on the food materials. Exotoxins can produce illness even when the microbes that produced them have been killed(Verma *et al.*, 2012).

2.8. Antimicrobial Activities of Medicinal Plant Extracts on Pathogens of Foodborne Diseases

What is more, natural plant-origin, antimicrobial compounds have been investigated as alternatives to synthetic ones for preserving food quality, owing to their effectiveness against food spoilage and foodborne pathogens(Antolak *et al.,* 2017). The use of natural compounds from plants can provide an alternative approach against food-borne pathogens. The mechanisms of action of most plant extracts with antimicrobial activity have been poorly studied(Sánchez *et al.*, 2010).

2.9. Minimal Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which prevents visible growth of bacterium. MIC depends on the microorganism, the affected human being (in vivo only), and the antibiotic itself. The MIC is determined by preparing solutions of the chemical in vitro at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and measuring the results using agar dilution or broth microdilution. Results have been graded into susceptible (often called sensitive), intermediate, or resistant to a particular antimicrobial by using a breakpoint. Breakpoints are agreed upon values, published in guidelines of a reference body, such as the U.S. Clinical and Laboratory Standards Institute (CLSI), the British Society for Antimicrobial Chemotherapy (BSAC) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). There have been major discrepancies between the breakpoints from various European countries over the years, and between those from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the US Clinical and Laboratory Standards Institute (CLSI). While MIC is the lowest concentration of an antibacterial agent necessary to inhibit visible growth, minimum bactericidal concentration (MBC) is the minimum concentration of an antibacterial agent that results in bacterial death. The closer the MIC is to the MBC, the more bactericidal the compound.

The first step in drug discovery is often the screening of a library drug candidate for MICs against bacteria of interest. As such, MICs are usually the starting point for larger pre-clinical evaluations of novel antimicrobial agents. Minimal inhibitory concentration (M.I.C.) values as determined by an agar-plate-dilution method for 60 bacterial isolates, consisting of Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus of animal origin against 20 antimicrobial drugs are presented. Of all the drugs, gentamicin had the best in vitro antibacterial activity in terms of M.I.C. when considering all the species of organisms together, while spectinomycin had the least activity(Hariharan *et al.*, 1974).

3. Materials and Method

3.1. Description of the Study Area

The study was conducted in Seka Chekorsa District of Jimma Zone, southwest Ethiopia. Seka Chekorsa is one of 18 districts in Jimma zone known for its predominant agronomy. Seka Chekorsa is located on 368 km away from the capital Addis Ababa and 18 km from Jimma town. Seka Chekorsa is bordered on the south by the Gojeb River which separates it from the Southern Nations, Nationalities and Peoples Region, on the west by Gera, on the northwest by Gomma, on the north by Mana, on the northeast by Kersa, and on the east by Dedo (Figure 2)(Melaku, 2016). Total population for this district was 208,096, of whom 104,758 were men and 103,338 were women; 7,029 or 3.38% of its population were urban dwellers. The majority of the inhabitants were Muslim (86.66%) followed by Orthodox Christian (10.93%), and Protestant (2.27 %)(Yigezu *et al.*, 2014).

The altitude of this district ranges from 1580 to 2560 meters above sea level; perennial rivers include the Abono, Anja, Gulufa and Meti. A survey of the land in this district shows that 45.3% is arable or cultivable (44.9% was under annual crops), 6.1% pasture, 25.8% forest, and the remaining 22.8% is considered swampy, degraded or otherwise unusable. Khat, peppers, fruits and teff are important cash crops. Coffee is another important cash crop for this district; over 50 square kilometers are planted with this crop(Yigezu et al., 2014). The district receives rainfall, about 1543.5 mm per annum. The average minimum and maximum daily temperatures of the area are 8.1 ^{0}C and 30.5 ^{0}C, respectively (Terefa, 2017).

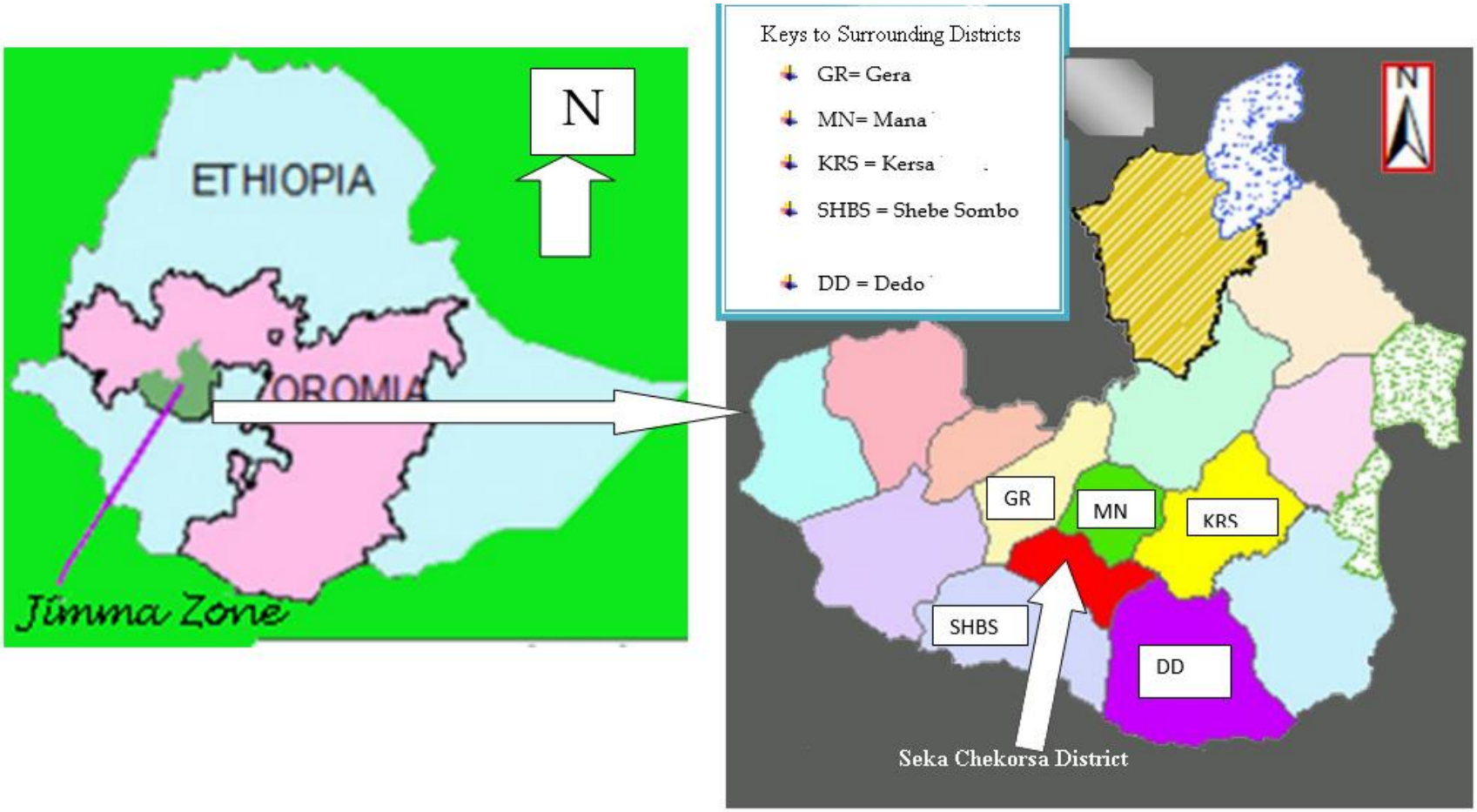

Figure 1: Map of the Study Site (Yigezu *et al*., 2014)

3.2. The Study Design

The study was conducted by using experimental and cross-sectional study. The cross-sectional study was conducted to assess medicinal plants from the study area. Following that, experimental design used to evaluate the antimicrobial and food preservative activities of the medicinal plants.

3.3. Sample Size Determination Technique

The technique used to select the sample from the medicinal plant practitioners was purposive technique. The number of available vendors or practitioners in the district are not large in number according to preliminary information obtained from the health officers of Seka Chekorsa district. Totally 47 respondents were participated in the study. Since the numbers of medicinal plant practitioners in that district are few, all well-known traditional medicinal plant venders in Seka Chokorsa from the study are, were participated in the study.

3.4. Data Collection Tools

Interview (as major tool) and questionnaires (Annex 1) were used for data collection. Questionnaire was developed to investigate what types of medicinal plants are currently being used by the practitioners in Seka Chokorsa district for the treatment of various diseases (Annex 1). Both interview and questionnaire contains detail information regarding the nature, source and identity of the herbs as well as their therapeutic usages, part(s) used, and mode of preparation. The used questionnaire was developed in English and translated to Afaan Oromoo.

3.5. Collection and Preparation of Plant Materials

After prioritizing depending the frequency from the respondents on the medicinal plants from the study area, six plants were selected for antimicrobial and food preservative activities (Table 1). After deciding the those plants, parts of those plant which have medicinal value had been collected and transported to Jimma University, College of Natural Science, Department of Biology, and Microbiology research lab.

Table 1: Lists of Medicinal Plants Selected for the Study Purpose

Scientific name of the plant	Local name (Afaan Oromoo)	Family name	Part used	Remark
Croton macrostachyus	Bakkanniisa	Euphorbiaceae	Leaf	Selected for antimicrobial activity
Zingiber officinale	Zinjibilii	Zingiberaceae	Underground stem	
Calpurnia aurea	Ceekaa	Fabaceae	Leaf	
Vernonia myriantha	Reejjii	Asteraceae	Leaf	
Moringa oliefera	Moringaa	Moringaceae	Leaf	Selected for their safety issue
Allium sativum	Qullubbii adii	Amaryllidaceae	Bulb	

After arriving in the lab all collected parts of the plants were washed gently by using distilled water to reduce microbial load on the plant and other chemical contamination on plant surface.

After washing all collected plant parts, it allowed to dry in dark area in the laboratory. The plant parts became dried within two weeks. To make it ready for extraction, the dried plant materials were grinded very well by using coffee grinding machine. During grinding the cross contamination of the plant parts were considered and the machine was cleaned very carefully while grinding different plant parts. After completion of grinding plant parts, the powders were stored separately in safe place until extracted. The same procedure was used for all plants used in this study (Sahraei *et al.*, 2014).

3.6. Extraction of Antimicrobials from Medicinal Plants

Prior to testing, an extract of the herbal material was prepared, using a rapid extraction process(WHO, 1998). Acetone, methanol and chloroform were used for extraction purpose for comparison of activity of crude extracted by solvents of different polarity (Elisha *et al.*, 2017). After extraction of selected medicinal plants, phytochemical screening of all crude was conducted (Elisha *et al.*, 2017). The Extraction was done by using maceration method. After getting the extracts by different solvents (i.e. acetone, methanol and chloroform), the filtrate was

evaporated using rotary evaporator to remove the solvent. Then the residue was re-dissolved in DMSO to antimicrobial activity of this extract (WHO, 1998). Following the same procedure three different plants were extracted.

After preparing the *Croton macrostachyus* powder, three different flasks were cleaned gently and dried very well. Then 40 gram of *Croton macrostachyus* powder was weighed and soaked in 150 ml of chloroform. In the second flak 40 gram of *Croton macrostachyus* was weighed and soaked in 150 ml of acetone. In the third flask 40 gram of *Croton macrostachyus* powder was weighed and soaked in 150 ml of methanol. After soaking in all three flasks with individual solvents, all flaks were shake gently by interval of six hour. On the third day of soaking, solution in all flaks were filtered by using filter paper. After getting only pure liquid filtrate, the filtrate was evaporated on a water-bath for as long as it is required to remove the solvent. Then the residue was re-dissolved in DMSO (WHO, 1998). All the three plant extraction also conducted following the same procedure as indicated in the following figure 2.

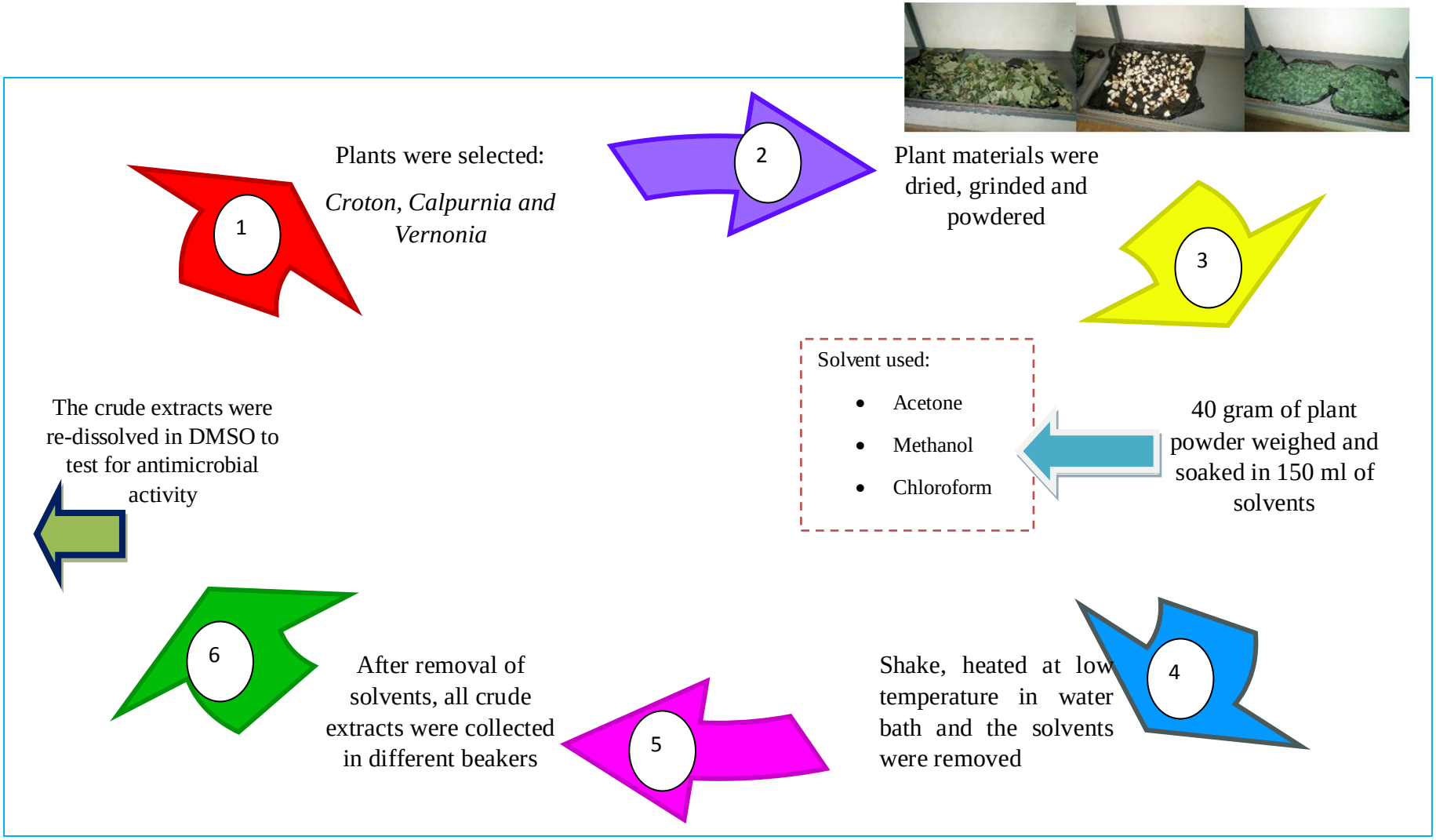

Figure 2: Extraction Procedure of Selected Medicinal Plants

3.7. Identification of Selected Medicinal Plants

All medicinal plants used in this study were collected from Seka Chekorsa district of Jimma Zone, Southwest Ethiopia. The altitude of this district ranges from 1580 to 2560 m above sea level. To identify the plants, it was transported to Jimma University, Department of Biology Herbarium. Currently, the plants were stored in this Herbarium after being identified by Dr. Dereje Denu, Assistant Professor of Botany at Jimma University Department of Biology and the voucher number was given and currently available in Jimma University, Department of Biology Herbarium(Figure 3).

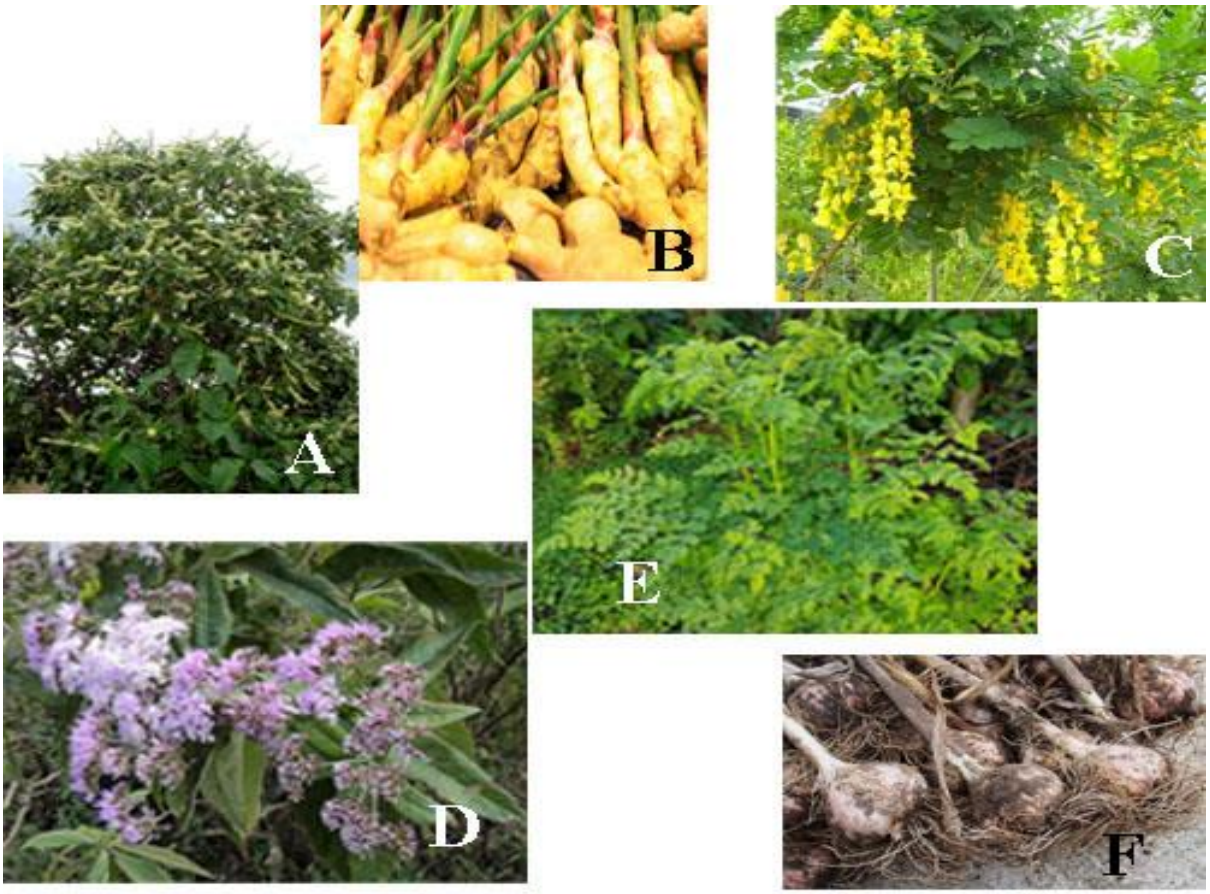

Figure 3: Description of Selected Medicinal Plants

(A) *Croton macrostachyus, (B) Zingiber officinale (C) Calpurnia aurea (D) Vernonia myriantha (E) Moringa oliefera (F) Allium sativum*

3.8. Isolation and Characterization of Foodborne Pathogens

3.8.1. Identification of the Isolates

Four different bacterial strains were isolated from the different foods. Those bacterial strains are: *Bacillus cereus, S. aureus, E. coli and S. Typhi*. As representative from fungal strains *Candida albicans* was used for antimicrobial activity tests. Chopped meat was taken from restaurant by using sterilized plastic bag.

3.8.1.1. Identification Test for *Staphylococcus aureus*

Two hundred fifty gram of chopped meat samples purchased from **Medhanialem Restaurant** [i] in Jimma city and transported to the laboratory in sterilized plastic bag. Then 25 gram of meat was weighed and suspended in 225 ml of sterilized Buffered peptone water. Then it was serially diluted from dilution factor 10^{-1} up to 10^{-5} and all diluted samples were inoculated to nutrient agar plates(Paramesh *et al.*, 2018; Pumipuntu, Kulpeanprasit *et al*., 2017). After incubation for 24 hr at 37 ^{o}C, gram staining and catalase test were conducted on young colonies on the nutrient agar. Then colonies with gram positive, cocci and catalase positive were transferred to nutrient broth to enrich it for 24 hr. After enriching it for 24 hr, the isolates were spread on Mannitol Salt Agar the selective media of *S. aureus* for further study(Gulani, 2016). Following that, the plates were incubated at 37 ^{O}C for 24 hours. After 24 hours incubation, golden yellow color colonies were detected as Staphylococcus on MSA and considered as presumptive for *S.aureus*.
Golden yellow colonies on MSA plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37 ^{o}C for 24 hrs for further purification. Then, a loopful of culture from the nutrient broth was streaked on nutrient agar supplemented with 0.75% NaCl and again incubated at 37 ^{o}C for 24 hrs.

Finally, the distinct colonies were characterized using the established microbiological methods. Gram-positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase and coagulase tests)(Nemo *et al.*, 2017). From the colonies of the Staphylococcus isolates, the colonies that showed coagulation after inoculating its broth into blood plasma was confirmed as *S.aureus* and used for the antimicrobial activity(Gulani, 2016; Pumipuntu et al., 2017).

3.8.1.2. Identification Test for *Eschercheria coli*

Two hundred fifty gram of chopped meat was purchased from **Medhanialem Restaurant** in Jimma city and transported in sterilized plastic bag to the laboratory. Immediately on arrival at the laboratory, from the sample 25 gram was aseptically weighed and suspended in 225 ml of sterilized buffered peptone water. After homogenizing, it was diluted by different dilution factors. Then all diluted sample were spread over nutrient agar plates(Solanki *et al.*, 2019). After incubation for 24 hr at 37 ^{o}C, different colonies of bacteria were detected on the nutrient agar. From the detected, the colonies with gram negative, rod shaped, non-spore forming and catalase negative were selected and enriched in nutrient broth (Humam, 2016; Islam *et al.*, 2014). After enrichment for 24 hr, the isolates transferred to MacKonkey Media the selective media of *Eschercheria coli*. After growing them on MacKonkey Agar Media all biochemical tests and morphological characterization suggested by (Al-baer *et al.*, 2017) were done including lactose fermentation and production of pink colonies on Macconkey agar (Paramesh *et al.*, 2018).

3.8.1.3. Identification Test for *Bacillus cereus*

Two hundred fifty gram of chopped meat was purchased from **Medhanialem Restaurant** in Jimma city and transported in sterilized plastic bag to the laboratory. Immediately on arrival at the laboratory, 25 gram of the meat was aseptically weighed and suspended in 225 ml of sterilized peptone water. After homogenizing, serial dilution was performed in buffered peptone water by dilution factor of 10^{-1} up to 10^{-6}. Additionally, all serially diluted samples were heated in water bath at 80 OC to decrease the probability of presence of other bacteria rather than *Bacillus* (Abraha, Bikila, Alemu, & Muktar, 2017). After cooling it, all diluted sample was spreader over nutrient agar plates(Solanki et al., 2019). After incubation at 37 oC for 72 hr the colonies that formed on the plates were tested for gram staining, catalase, endospore formation and their shape. After performing this activities gram- positive, catalase positive, rod-shape, spore-forming bacterial colonies were enriched in nutrient broth for 24 hr (Abraha *et al*., 2017). Following the enrichment all suspected isolates were transferred to Bacillus cereus Agar Base the selective media of *Bacillus cereus* (El-arabi *et al.*, 2013) for further study. Following that, the plates were incubated at 35 ^{O}C for 72 hours. After 24 hours the colony of *B. cereus* were

detected. The suspected colony of *Bacillus cereus was* determined by the surface plating method with mannitol egg yolk polymyxin (MYP) agar (Solanki et al., 2019). Typical colonies of *B. cereus* were then transferred to nutrient agar slants and identification was confirmed by microscopic and biochemical characterization that includes Gram staining, lecithinase production, citrate taste, motility, endospore formation and hemolysis(Abraha *et al*., 2017).

3.8.1.4. Identification of *Salmonella Typhi*

Two hundred fifty gram of **meat firfir** [ii] was taken from **Madhanialem Restaurant** and aseptically transported to the lab. Buffered Peptone Water, was prepared. Then 225 ml of the medium was dispensed into erliminiyer flask. Rappaport-Vassiliadis was prepared for Salmonella Enrichment Broth following product label. Twenty five gram of the test specimen (firfir) was added to 225 ml of Buffered Peptone Water and incubated at 35℃ for 24 hr hours. Following that, 0.1 ml of the pre-enrichment Buffered Peptone Water was inoculated to 10 ml of Rappaport Vassiliadis. Then Salmonella Enrichment Broth was incubated at 37 ℃ for 24 hours.

After incubating for 24 hr it was sub-cultured on XLD Agar and incubated at 37℃, the plates were examined for growth at 18 – 24 hours. Then rod-shaped Gram negative, non-lactose fermenting, non-sporing (Oluyege *et al.*, 2015) colonies with black centres on Xylose Lysine Desoxycholate Agar (XLD)(Salm-surv *et al.*, 2015) were selected and different biochemical and morphological identifications were conducted(Oluyege *et al*., 2015; Salm-surv et al., 2015).

3.8.2. Procedure for Biochemical and Other Characterization

3.8.2.1. Cell Morphology

Gram staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 96 % of ethanol for 20 seconds and washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained by safranin and dried by absorbent paper. Finally, the air-dried smear was observed under oil immersion objective. At the completion of the Gram Staining, gram-negative bacteria were stained pink/red and gram-positive bacteria were stained blue/purple(Gram, H, 1884).

Shape

Shape of bacterial cell was decided after conducting gram staining and observation under microscope by busing 100X objective.

Endospore test

A smear of isolates was prepared on a clean glass slide and allowed to air-dry. The air-dried smear was heat fixed. Heat fixed smear was flooded with 0.5 % (w/v) malachite green solution and steamed using cotton dipped in 96 % ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for 30 seconds. The slide was washed with tap water and air dried/ blotted to be observed under the oil immersion lens (×1000) to check the presence of endospore(Shields *et al.*, 2012).

Motility test

A motility medium was prepared using a test tube. A purified broth culture was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium to the bottom of the tube and incubated at 35°C for 24 hours. A positive motility test was indicated by a red

turbid area diffusing away from the line of inoculation and a negative test was indicated by red growth along the inoculation line only but no further.

3.8.2.2. Biochemical Tests

KOH Test

Two drops of 3 % KOH solution were placed on a clean microscopic slide. A colony was aseptically picked from the surface of nutrient agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution become viscous, the thread of slime followed the loop for 0.5 to 2 cm or more in gram-negative bacteria. In case of no slime and a watery suspension did not follow the loop, the reaction was considered negative and the isolate was considered as gram positive bacteria.

Starch hydrolyses test

Using a sterile technique, a single streak inoculation of organism to be tested was made into the center of labeled plate. Bacterial inoculated plates were incubated for 48 hours at 37°C. Following incubation, the surface of plate was flooded with iodine solution by a dropper for 30 seconds. The excess iodine was poured off. Then after examining for the clear zone around the line of bacterial growth, the formation of clear zone shows positive because of the bacterial hydrolyzed the starch no blue black was formed. If, there is formation of blue black around bacterial colony it shows negative because no starch was hydrolyzed.

Citrate utilization test

The citrate agar slant was streaked and the tube was incubated at 37 ^{o}C for 24 hrs to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive (Gulani, 2016)

Catalase test

Catalase test was carried out after young colonies flooded with a 3% solution of H_2O_2. The formation of bubbles indicated the presence of catalase.

Coagulase

First 1 ml of plasma was diluted in 10 ml of saline solution prepared. Three small test tubes and label as T (Test), P (Positive Control) and N (Negative Control). Test was 18-24 hr broth culture, Positive control is 18-24 hr Standard strain of *S. aureus* (ATCC 25923) broth culture and Negative control is sterile broth. Then 0.5 ml of the diluted plasma was pippeted into each tube. After mixing, incubate the three tubes at 37 ^{o}C. After examining for clotting after 6 hr, formation of clotting in isolate (test) accepted as the isolate was *S. aureus*(Nikolina *et al.*, 2017; Padilha *et al.*, 2000).

Lecithinase test

Mannitol Egg Yolk Polymixin (MYP) agar was prepared from its ingredients and sterilized. Aseptically it was transferred to plate. Then the agar plate divided into three equal parts. Loopful of culture from the first nutrient broth of the suspected *B. cereus* taken and streak on the medium to obtain isolated colonies. Then it was incubated at 37 ^{O}C for 48 hr. After 48 hr examine the color change in the medium and opacity zones around colonies presumed as presence of *B. cereus*(Use transmitted light to observe the halo)(Vlab.amrita.edu, 2011)

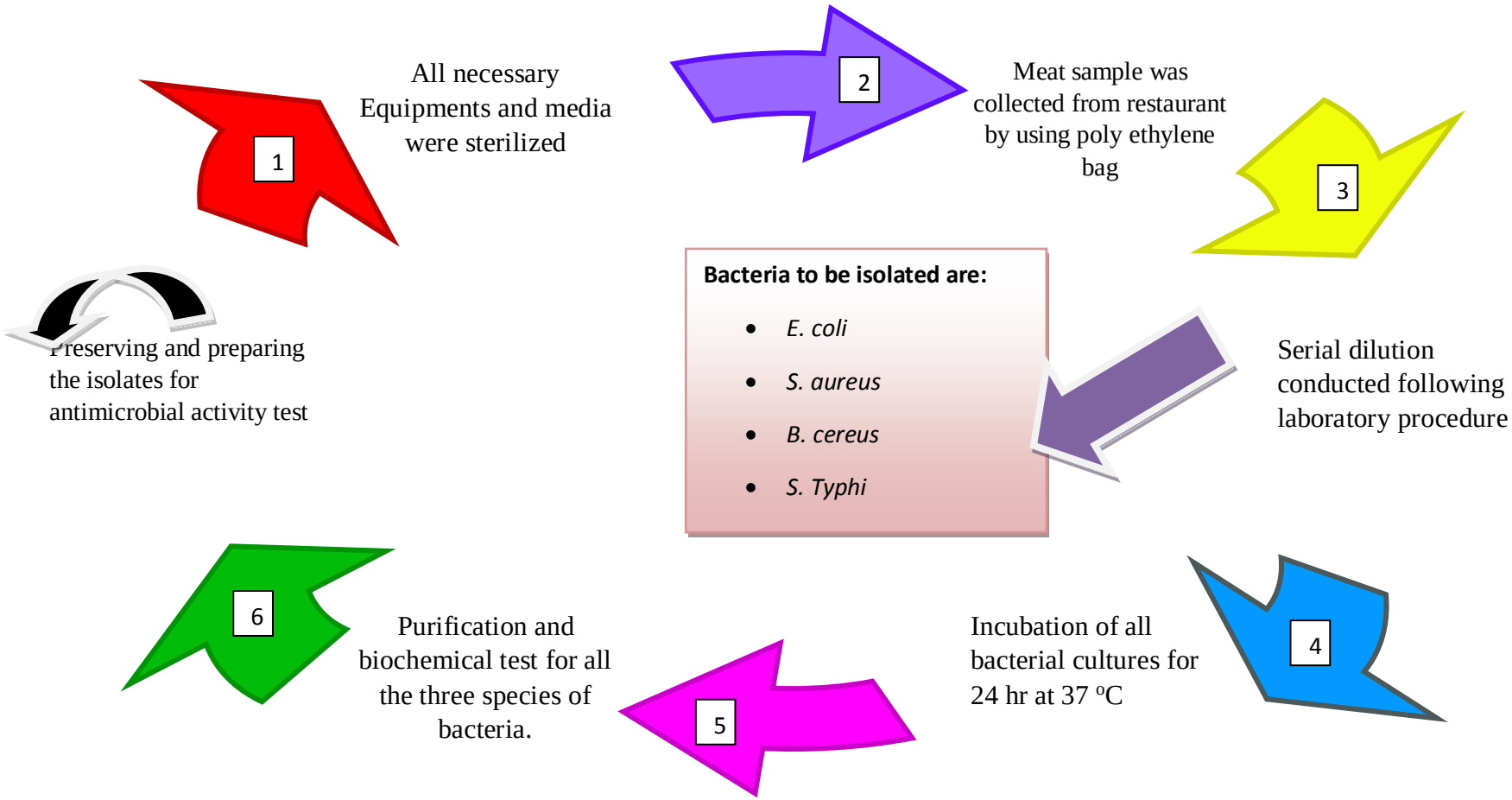

Figure 4: Isolation, Purification and Characterization of test microorganisms from meat

3.8.3. Preparation of Inoculants

In this study both preserved isolates and standard strains (*S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922)) were used as test microorganisms. The preserved isolate of the four bacteria were activated and used for antimicrobial activity test. Bacterial strains were grown to exponential phase at 37℃ for 24h and diluted by sterile peptone water to a final density of 10^7~10^8 CFU/ml adjust the cell density using 0.5 McFarland standard (Kuang, Zhang, Qi, Wu, & Liu, 2012). The strains of those pathogenic microorganisms were activated by using appropriate and standard nutrients. *E. coli*, *S. aureus*, *B. cereus* and *S. Typhi* were used for antimicrobial activity test (Simpson et al., 2008). From fungal species *C. albicans* was used.

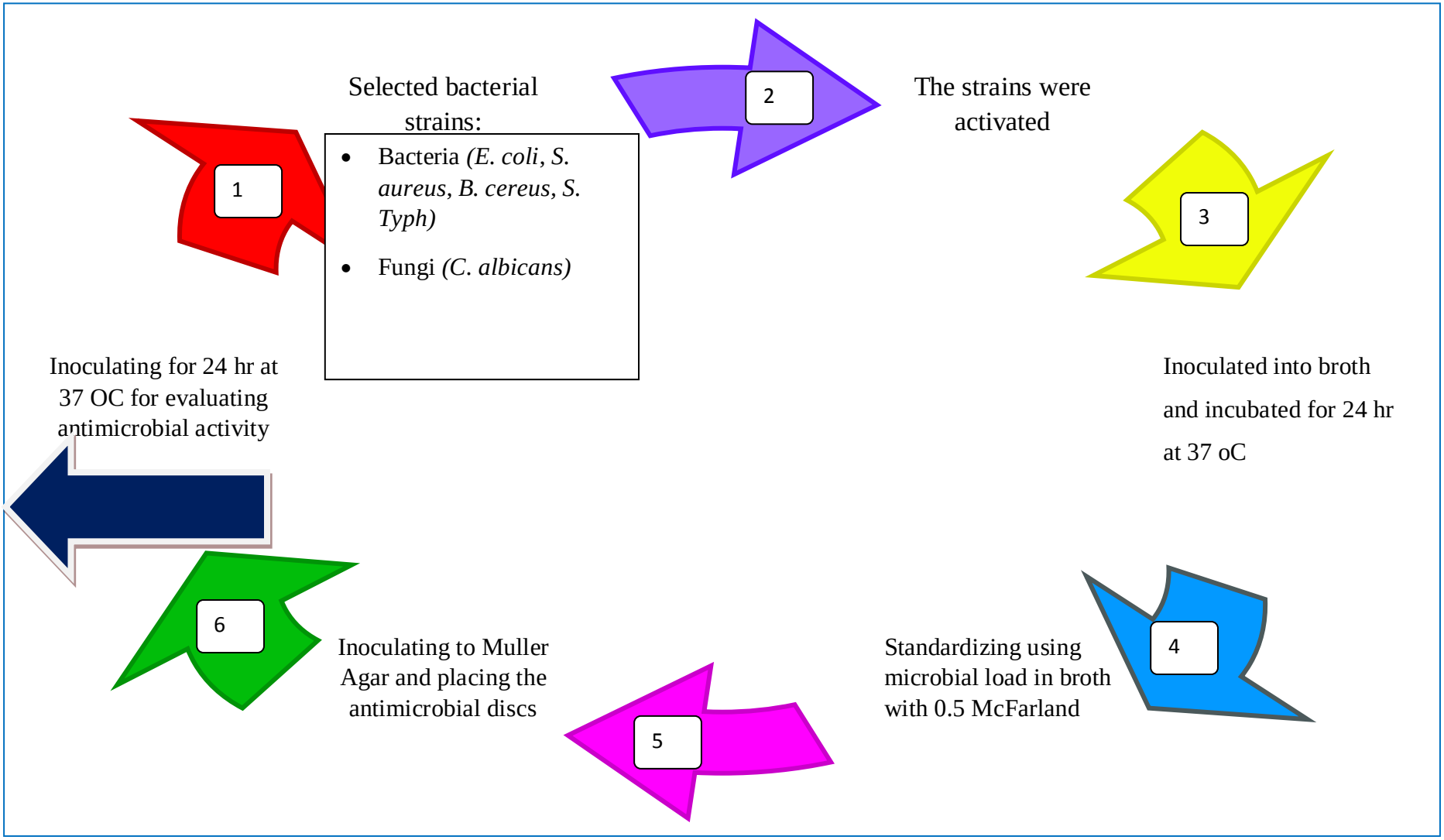

Figure 5: Brief description of inoculums preparation

3.9. Antimicrobial Activity Tests of Medicinal Plant Extracts

Four isolates of bacteria were used as test microorganisms. In parallel with standard reference, three bacterial strains; *E. coli, S. aureus and B. cereus* were isolated from chopped meat that was taken from hotel (Kuang *et al.*, 2012). Disc of diffusion method was used to determine antimicrobial activity of plant extracts of selected medicinal plant. A circular filter paper with diameter of 6 mm was placed on plate to measure inhibition zone (Kuang *et al.*, 2012).

Diffusion disk was manually prepared from filter paper by using paper punch. The diameter of the disk prepared from filter paper was 6 mm. After preparing enough diffusion disks, the disks were sterilized by using. Every time while performing antimicrobial activity the diffusion disks were sterilized. Crude extract of the three medicinal plants (i.e. *C. microstachyus*, *C. aurea* and *V. myriantha*) were re-dissolved in DMSO (Dimethyl Sulfoxide) as the following (Table 2).

Table 2: Mass of re-dissolved plant crudes that extracted by different solvents

Scientific name of the plant	Solvent used	Mass of crude extract	Volume of DMSO
Croton macrostachyus	Chloroform extracted	0.5 gram	1 ml
	Acetone extracted	0.5 gram	1 ml
	Methanol extracted	0.74 gram	1 ml
Calpurnia aurea	Chloroform extracted	0.74 gram	1 ml
	Acetone extracted	0.41 gram	1 ml
	Methanol extracted	0.98 gram	1 ml
Vernonia myriantha	Chloroform extracted	0.5 gram	1 ml
	Acetone extracted	0.5 gram	1 ml
	Methanol extracted	0.5 gram	1 ml

After dissolving the crude extracts in DMSO, the disks of diffusion were put in solution of crude extract. To increase the absorption of the crude solution to the disks, they were stayed in the solution for thirty minutes.

3.9.1. Antimicrobial Activity Tests

For current study strains of ***E. coli, S. aureus, B. cereus*** and *S. Typhi* were used as test microorganisms from bacteria while while *C. albicans* used from fungal strain(Kuang et al., 2012). To compare the standard strains with isolated strains, isolation of four species of bacteria was conducted. Those species of bacteria are: *E.coli, S. aureus, B. cereus* and *S. Typhi*. After activating both isolate and standard strain the antimicrobial activities of crude extract from different type of solvents was tested.

After testing antimicrobial activities of all crude extracts from all solvents, the inhibition diameter of both isolated strains and standard strains will be compared with each other. In addition to plant extracts common antibiotics known for those strains ciproflavin was used for comparison to plant extracts. Therefore, antimicrobial activities of all extracts from different solvents were compared with recommended antibiotics.

Table 3: Dissolved crude extract during antimicrobial activity test

	Dissolved crude						Controls		
	Croton Chloroform extracted	Crotone Acetone extracted	Croton Methanol extracted	Calpurnia chloroform	Calpurnia Acetone extracted	Calpurnia Methanol extracted	Euro acetone extracted	Ciproflavin disc (Positive)	DMSO (Negative)
Mass of dissolved crude in g	0.58	0.5	0.5	0.74	0.41	0.98	0.5		
Test strains	*S. aureus, B. cereus, E. coli, S. Typhurium and C. albicans*								

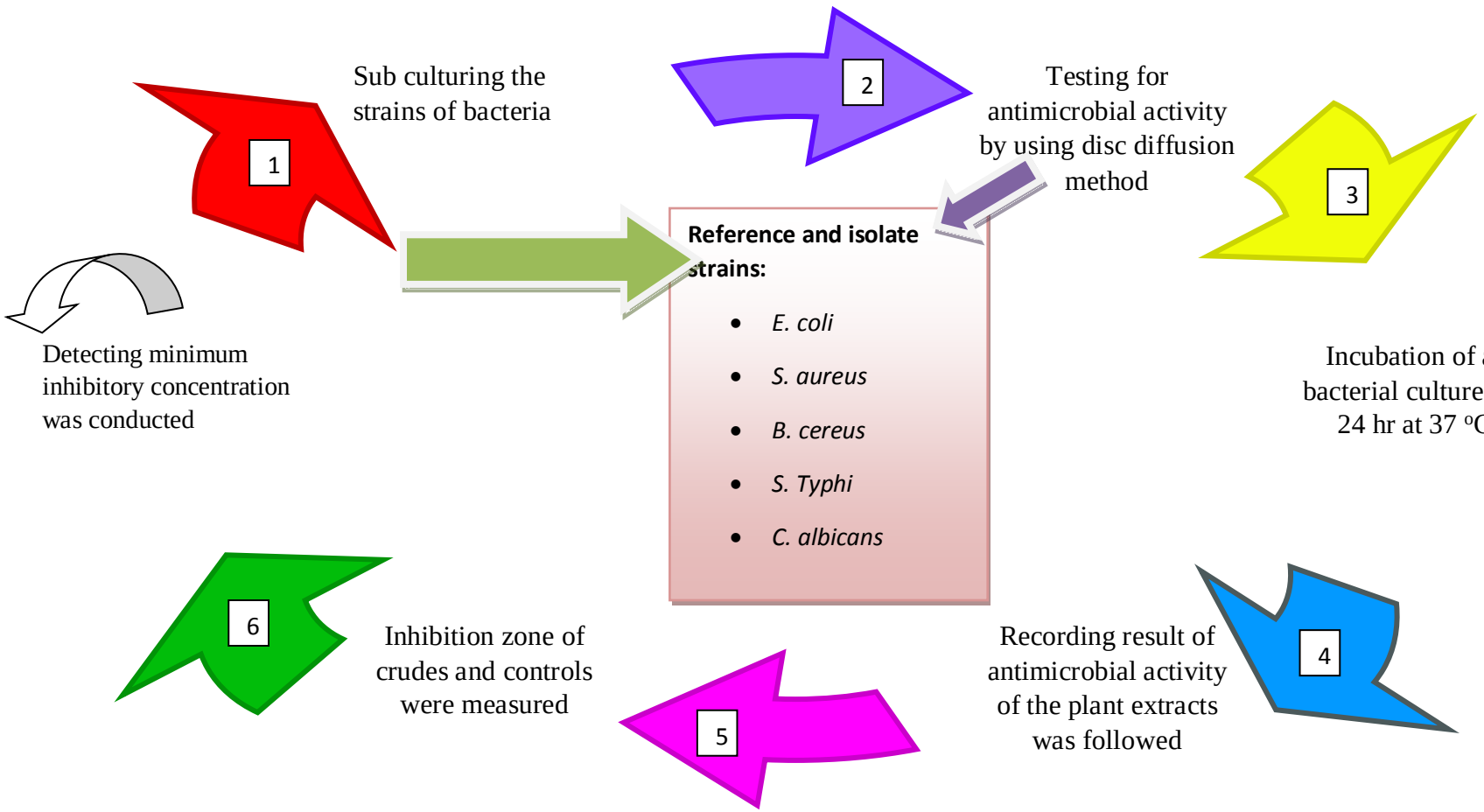

Figure 6: Procedure followed during antimicrobial activity test

3.9.2. Minimum Inhibitory Concentration of Antimicrobial Activity Tests

Minimum inhibitory concentration of the effective crude extracts were done by using the following procedures. To get different data each crude diluted at different folds and the mean of MIC was taken. As shown in the following table (Table 4), it was diluted by starting from half up to one sixth folds of the first antimicrobially active concentration.

Table 4: Steps followed to detect minimum inhibitory concentration

Bacterial strain	Plant and solvent used	Measure of g/ml of DMSO
Bacillus cereus	*Croton macrostachyus* Chloroform extracted	1 g/1ml
		0.58 g/ 1ml
		0.29 g/1ml
		0.19 g/1ml
		0.135g/1ml
Staphllococus aureus	*Croton macrostachyus* chloroform extracted	1g /1ml
		0.58 g/ 1ml
		0.29 g/1ml
		0.19 g/1ml
		0.135g/1ml
	Croton macrostachyus acetone extracted	1g/1ml
		0.46g/1ml
		0.23g / 1ml
		0.153g / 1ml
		0.115g / 1ml
	Croton macrostachyus methanol extracted	1.5 g/1ml
		0.72g /1ml
		0.36g / 1ml

		0.253g /1ml
		0.19g / 1ml
	Calpurnia aurea acetone extracted	0.41g / 1ml
		0.205g / 1ml
		0.136g / 1ml
		0.102g / 1ml
Salmonella typhurium	*Calpurnia aurea* methanol extracted	2 g/1ml
		0.98g /1ml
		0.49g / 1ml
		0.326g /1ml
		0.245g / 1ml
Candida albicans	*Croton macrostachyus* Chloroform extracted	1 g/1ml
		0.58 g/ 1ml
		0.29 g/1ml
		0.19 g/1ml
		0.135g/1ml

After incubating the inoculated Muller agar media the zone of inhibition of all crude extracts were recorded. Based up on the record of the zone of inhibition the minimum inhibitory concentration was determined.

3.10. Phytochemical Screening of the Extracts of Selected Medicinal Plants

Preliminary qualitative phytochemical screening was carried out with the by following method used by (Mulata *et al.*, 2015). The phytochemical screening of both Croton and Calpurnia were conducted the same procedure as follows.

To test for Steroids, 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of

steroids (Gibbs RD, 1974). **To test for Terpenoids**, 1ml of extract was added to 2 ml of acetic anhydride and concentrated H_2SO_4. Formation of blue or green rings indicates the presence of terpenoids. **To test for Tannins**, 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

To test for Saponins, 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

To test for Anthocyanins, 2 ml of aqueous extract is added to 2 ml of 2N HCL and ammonia. The appearance of pink-red turn's blue-violet, indicates the presence of anthocyanins.

To test for Flavonoids, extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids. **To test for Alkaloids**, 0.2 gram of extracts was added in a test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then took 5 ml of 2% HCL and poured in a test tube having the mixture of plant extract and hexane. Heated the test tube having the mixture, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

To test for Phenolic Compounds, to 2 gram of the extract of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1 % $FeCl_3$ and 1 ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green color indicates the presence of phenolic compounds. From all the crudes the effective crudes that showed good antimicrobial activity were screened for their phytochemical constituents.

3.11. Food Preservative Activity Tests

For this study fresh chopped meat was purchased from **Madhanialem Restaurant** in Jimma city and transported to Jimma University Microbiology Research Lab by using poly ethylene bag. After arriving in laboratory the microbial load of fresh meat sample was evaluated by using the plate count agar.

Then, two beakers were used to test for food preservative activity ginger. To evaluate the food preservative activity the plant powder was used. Two different beakers were taken. In the first beaker 10 gram of chopped meat was taken and 2 gram of ginger powder was added and gently mixed with meat by using sterilized glass road. After mixing with ginger powder the beaker was coved with sterilized aluminum foil. In the second beaker 10 gram of chopped meat was added and left without adding any medicinal plant powder to use it as control. Finally both beakers were covered with aluminum foil and put in dark place for 24 hr. After waiting for 24 hr both samples in the beakers were taken and microbial load of the food in each beaker was calculated. This activity was performed three times to compare the result of different times. Before applying the plant to food the microbial load of fresh food before application of the extract was counted to evaluate food preservative activity after applying the plant powder. In every activity the mass of taken food in beaker and amount of extract volume was measured *(Elisha et al., 2017; Verma et al.,* 2012; WHO, 1998). Then the load of microbe in the food before and after application of plant powder was used for evaluating food preservative activity of plant powder (Verma *et al.,* 2012). Food preservative activity of the Garlic and Moringa also performed following the same procedures.

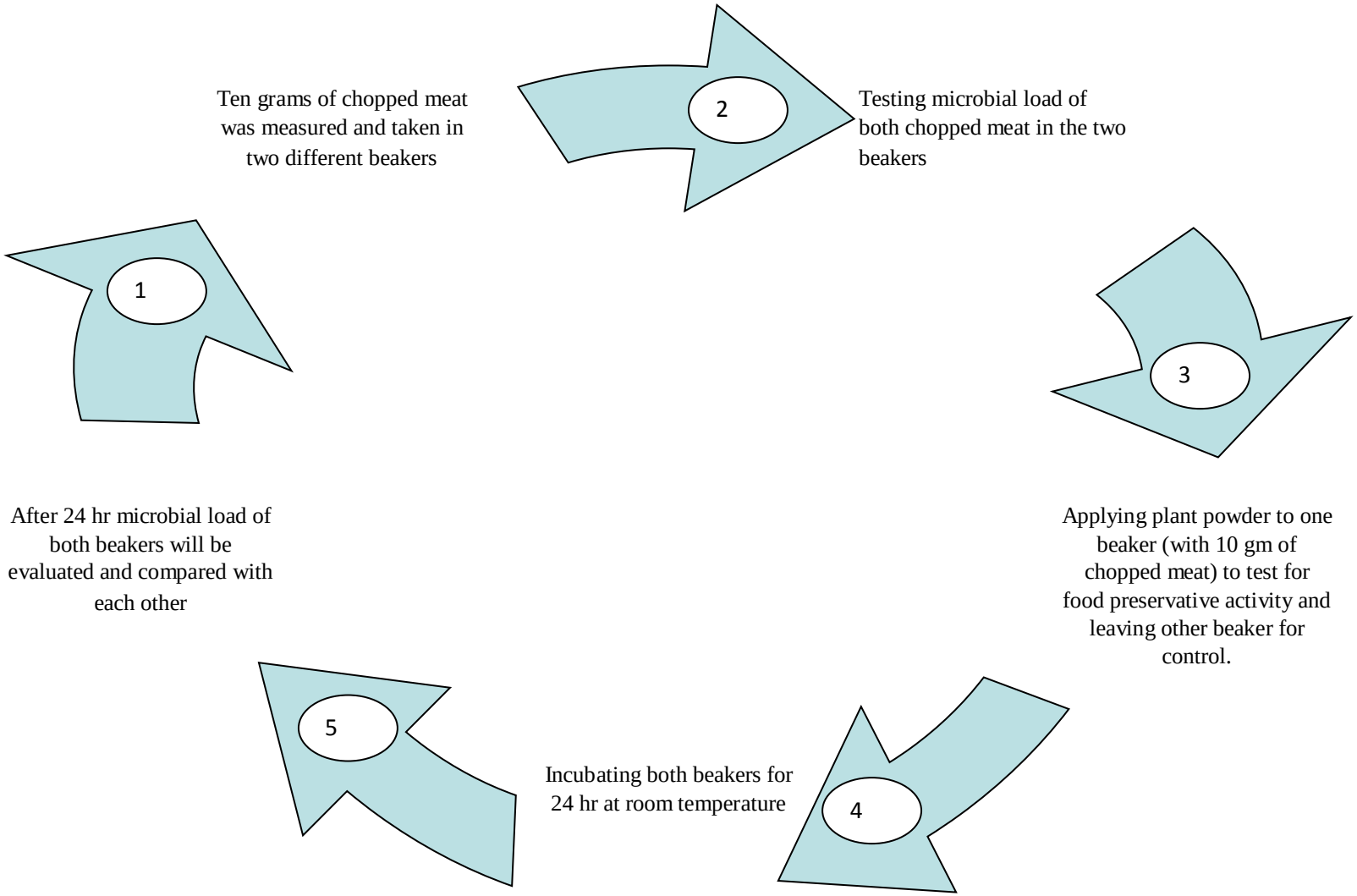

Figure 7: Steps Followed to Test Food Preservative Activity of Plant Powders

3.12. Data Analyzing Method

This study includes both qualitative and quantitative method. Mostly qualitative method was used to detail expression of the data. The collected data was checked, edited and well organized before being analyzed. The data was analyzed by using descriptive statistics such as percentage and frequency and presented by using graphs and tables. Also there are parts which was analyzed by using SPSS software version 20.

4. Result and Discussion

4.1. Result

4.1.1. Socio-demographic Status of Practitioners

Most of the practitioners of medicinal plants (respondents) are elder. These shows that mostly the knowledge of medicinal plants is still in the hands of elders and it needs more scientific documentation. Therefore still it needs scholars' attention to do the documentation of this indigenous knowledge in the community. Additionally most of the respondents are male. Most of the practitioners of medicinal plants are from the rural area. Further information is described as showing in the following table (table 5).

Table 5: Socio-demographic Characteristics of respondents

Variable		**Frequency**	**Percentage**
Age	24-32	22	47.1
	33-40	19	41.2
	Above 40	6	11.8
	Total	47	100.0
Sex	Male	36	76.6
	Female	11	23.4
	Total	47	100.0
Practice of medicinal plants	Healer	38	80.9
	User	9	19.1
	Total	47	100.0
Literacy status	Illiterate	39	83.0
	Literate	8	17.0
	Total	47	100
Residence	Urban	6	12.8
	Rural	41	87.2
	Total		

4.1.2. Medicinal Plant List from the Respondents

Table 6: Medicinal Plant List Recorded from the Respondents

Disease name	Plants used to treat (Local name)/ In Afaan Oromoo	Plants scientific name	Family name	Part used	Preparation	The route of taking (i.e. oral, mouth,)	Number of respondents out of 47
Diarrhea	Sunqoo	*Trigonella foenugraceum*		Seed	Consuming raw seeds	Oral	21
	Ceekaa	*Calpura aurea*	Fabaceae	Leaf part	Drinking the prepared liquid	Oral	38
	Reejjii	*Vernonia myriantha*	Asteraceae	Leaf part	By drinking the liquid	Oral	22
	Bakkanniisa	*Croton macrostachyas*	Euphorbiaceae	Leaf	By drinking the prepared liquid.	Oral	31
Abdomen pain	Qullubbii adii + Macaafata gurraacha + Zinjibila			Bulb + seed + underground stem	By dissolving those grinded parts together and then drinking it.	Oral	43

Ascariasis	Waggartii			Leafy part	By drinking the liquid extracted.	Oral	15
	Bakkanniisa	*Croton macrostachyas*	Euphorbiaceae	Leaf	By drinking the prepared liquid.	Oral	31
Tonsillitis	Geeshee	*Bhamnus prinoides*		Leaf part	By drinking the grinded plant part.	Oral	13
	Hiddii			Fruit juice	By painting on the tonsillitis area gently.	Oral	15
	Goodarree	*Colocasia esculenta*		Leaf	By eating the boiled and cooled the tuber for about a week	Oral.	10
Wound and skin diseases	Bakkanniisa	*Croton macrostachyas*	Euphorbiaceae	Leaf and stem	By painting the liquid extracted from the leaf part	Applying on the wound and infection area	31

4.1.3 Frequently Used Medicinal Plants in the Study Area

After prioritizing the responses of practitioners from the study area the following five plants were selected for this study purpose.

Following prioritization and originality of the study *Croton macrostachyus, Calpurnia aurea and Vernonia myriantha* were selected for antimicrobial activity tests.

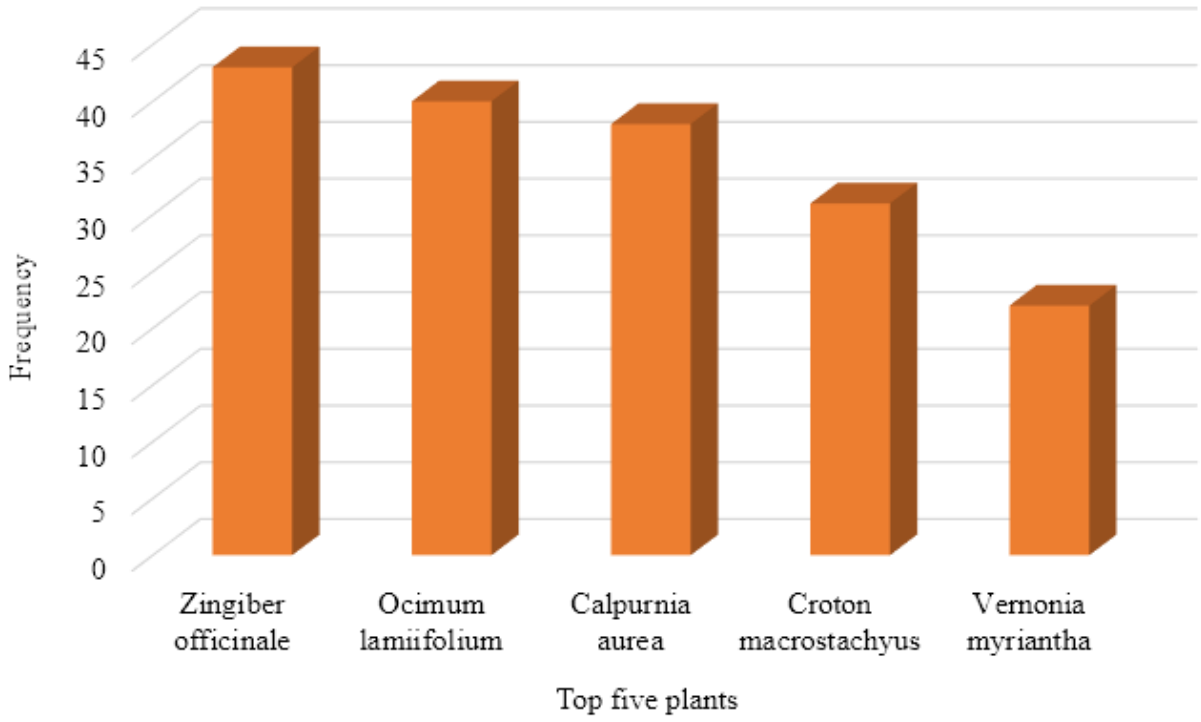

Figure 8: Top five most commonly used medicinal plants

4.1.4. Extraction of Collected Plant Samples

Table 7: Brief extraction procedure, mass of yield and mass dissolved crude extract

Plant name Scientific name	Solvents used in ml	Mass of powdered plant sample	Yield of plant extract in gram after three days soaking	Percent of yield
Croton macrostachyus	Chloroform (150 ml)	40 gram	1.14 gram	2.85%
	Acetone (150 ml)	40 gram	0.73 gram	1.85%
	Methanol (150 ml)	40 gram	1.6 gram	4%
Calpurnia aurea	Chloroform (150 ml)	40 gram	1.96 gram	3.025%
	Acetone (150 ml)	40 gram	1.21 gram	3.02%
	Methanol (150 ml)	40 gram	3.5 gram	8.75%
Vernonia myriantha	Acetone (150 ml)	40 gram	2.3 gram	5.75%

4.1.5. Phytochemical Screening of the Extracts of Selected Medicinal Plants

In this case phytochemicals of two plants that showed high antimicrobial activity (i.e. *Croton microstachyus* and *Calpurnia aurea* was performed. The following table contains all the phytochemical screening of the crude extracts. After performing phytochemical screening alkaloids, flavonoids, saponin, phenols, tannins, terpenes and steroids were detected in the crude of both plants.

Table 8: Phytochemical Screening of the Extracts of Selected Medicinal Plants

No.	Plant	Solvent used during extraction	Phytochemical screening test result							
			Alkaloids	Flavonoids	Saponin	Phenolic compounds	Tannins	Terpenoids	Cardiac glycosides	Steroid / Phytosterols
1.	*Croton macrostachyus*	Chloroform extracted	+	+	+	+	+	+	+	+
		Acetone extracted	+	+	+	+	+	+	+	+
		Methanol extracted	+	+	+	+	+	+	+	+
2.	*Calpurnia aurea*	Chloroform extracted	+	+	+	+	+	+	+	+
		Acetone extracted	+	+	+	+	+	+	+	+
		Methanol extracted	+	+	+	+	+	+	+	+

"+"Shows the presence

4.1.6. Isolation and Identification of Test Strains

After isolating test microorganisms, it was characterized by using morphological, biochemical and shape of those bacteria. Biochemical, morphological and endospore tests four strains of bacteria were conducted and the strains were preserved for antimicrobial activity tests. Those strains were, *S. aureus, B. cereus, E. coli* and *S. Typhi*. Additionally the strain of *C. albicans* also used as representative strain from fungi. As shown in annex (Annex 3) different biochemical tests were conducted to identify the bacterial strains.

To identify *S. aureus* from other staphylococci plasma coagulase test was done. The distinct colonies were characterized using the established microbiological methods. Gram-positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase and coagulase tests). From the colonies of the *Staphylococcus* isolates, the colonies that showed coagulation after inoculating its broth into blood plasma was presumed as *S. aurous* and used for the antimicrobial activity.

From the detected suspected colonies of *E. coli*, the colonies with gram negative, rod shaped, non-spore forming and catalase negative were presumed as *E. coli* suspect. Then it was identified by using lactose fermentation and production of pink colonies test on MacKonkey agar.

The suspected colony of *B. cereus was* determined by the surface plating method with Mannitol Egg Yolk Polymyxin (MYP) agar. Typical colonies of *B. cereus* were then transferred to nutrient agar slants and identification was confirmed by microscopic and biochemical characterization that includes Gram staining, lecithinase production, citrate taste, motility, endospore formation and hemolysis.

Following different biochemical tests, the rod-shaped gram negative, non-lactose fermenting, non-sporing colonies with black centres on Xylose Lysine Desoxycholate Agar (XLD) were presumed as *S. Typhi*.

4.1.7. Antimicrobial Activity of Selected Extracts of medicinal Plants

Croton macrostachyus that extracted by chloroform showed good antimicrobial activity. As shown is the following table (Table 10) it inhibited growth of *B. cereus* (12.33 mm), *S. aureus* (23.33 mm) and *C. albicans* (22.33 mm) by forming mentioned zone of inhibition.

Table 9: Antimicrobial Activity of Selected Extracts of medicinal Plants

Plants scientific name	Solvents used during extraction	Zone of inhibition (mean ± SD) in mm				
		E. coli	*B. cereus*	*S.aureus*	*S.typhyurium*	*Candida albicans*
Croton macrostachyus	Chloroform extracted	+	12.33 ± 2.87	23.33 ± 3.30	+	22.33 ± 1.70
	Acetone extracted	+	+	17.67 ± 2.05	+	19.67 ± 0.94
	Methanol extracted	+	+	12.67 ± 2.05	+	22.00 ± 0.82
Calpurnia aurea	Chloroform extracted	+	+	+	+	16.33 ± 0.94
	Acetone extracted	+	+	11.00 ± 1.41	+	15.67 ± 0.94
	Methanol extracted	+	+	+	15 ± 2.16	+
Vernonia myriantha	Acetone extracted		15.33 ± 2.49	NP	NP	NP
Control	+ve Control Ciproflavin disc	23	25	28	25	33.00
	DMSO					

"+" Showed antimicrobial activity but < 10 mm, "NP", Not performed

As shown in the below (figure 9) two standard strains *S. aureus* (ATCC 25923) and E. coli (ATCC 25922) were activated. After activating the strains the crude showed zone of inhibition as shown by the figure 9.

Additionally there is relationship with the result of isolate based antimicrobial activity and that of standard strains. *C. microstachyus* extracted with all the three solvents were tested on *S. aureus*. *C. aureus* extracted by acetone and *C. microstachyus* extracted by chloroform was tested on *E. coli*.

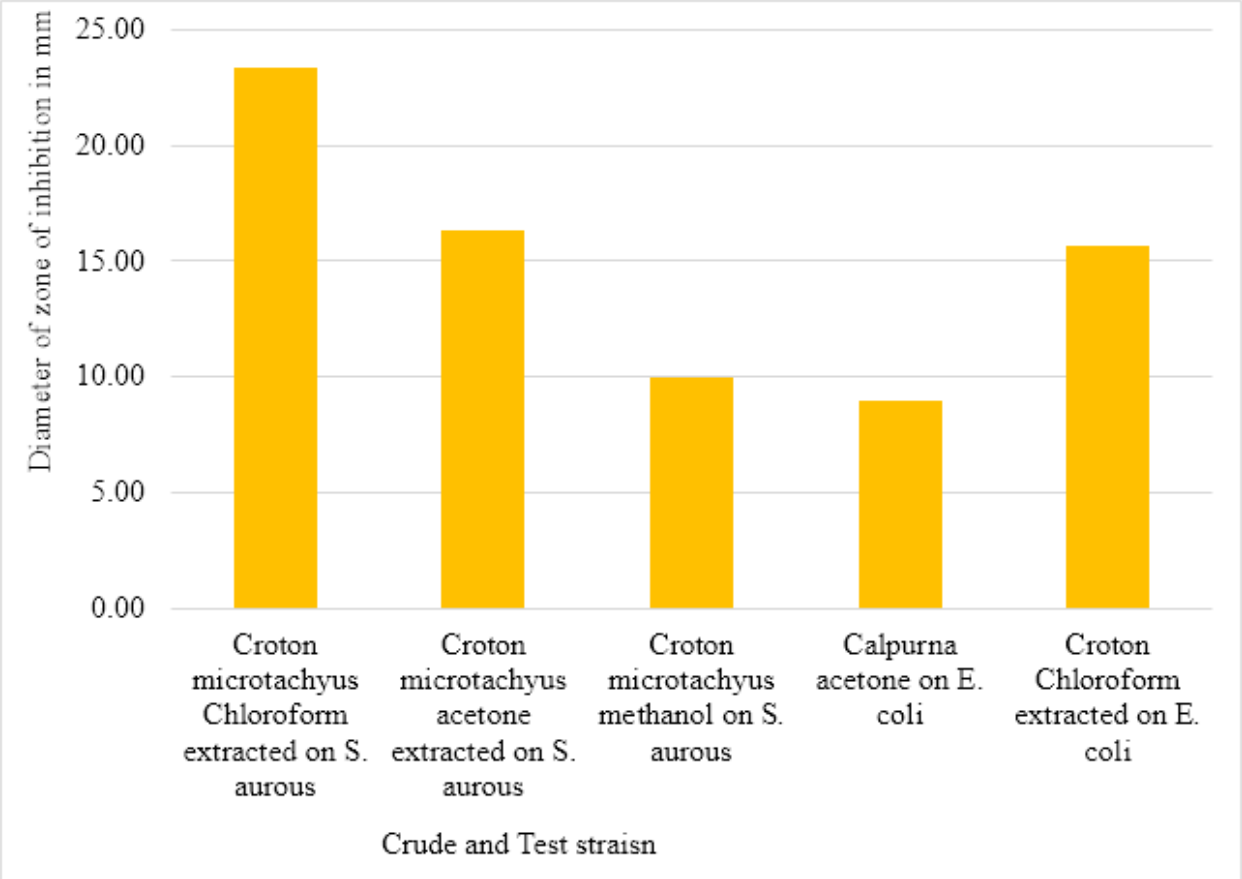

Figure 9: Antimicrobial activities of some crudes on Standard Strains of *S. aureus* and *E. coli*

4.1.8. Minimum Inhibition Concentration of Plant Crude Extracts

After conducting the activities by diluting the crude at different folds the mean of minimum inhibitory concentration was recorded as the following (Table 11).

Table 10: Result of minimum inhibitory concentration of the extracts on bacterial strains

Test strain	Plant and solvent used	MIC ± SD in gram/ml
Bacillus cereus	*Croton macrostachyus* Chloroform extracted	0.2675 ± 0.019
Staphllococus aureus	*Croton macrostachyus* chloroform extracted	0.235 ± 0.021
	Croton macrostachyus acetone extracted	0.245 ± 0.011
	Croton macrostachyus methanol extracted	0.365 ± 0.03
	Calpurnia aurea acetone extracted	0.13425 ± 0.004
Salmonella typhurium	*Calpurnia aurea* methanol extracted	0.4275 ± 0.033
Candida albicans	*Croton macrostachyus* chloroform extracted	0.1825 ± 0.015
	Croton macrostachyus acetone extracted	0.149 ± 0.003
	Croton macrostachyus methanol extracted	0.185 ± 0.011
	Calpurnia aurea chloroform extracted	0.3625 ± 0.038
	Calpurnia aurea acetone extracted	0.245 ± 0.018

4.1.9. Food Preservative Activity of Selected Medicinal of Plants

Three plants were tested for their food preservative activities. Those plants were ginger, moringa and garlic. In this study plant powder showed the ability to inhibit growth of microbial load in chopped meat sample used during the study.

Table 11: Log of microbial load of experimental and control in food preservative activities

Plant used as food preservative		Microbial load of fresh chopped meat.	Microbial load of control after 24 hr.	Microbial load of chopped meat mixed with plant powder.
Zingiber officinale	Mean ± SD	1.92 ± 0.29	6.08 ± 0.66	2.49 ± 1.30
Moringa oliefera	Mean ± SD	2.60 ± 0.67	3.54 ± 0.81	2.62 ± 0.33
Allium sativum	Mean ± SD	2.37 ± 0.25	3.60 ± 0.44	2.72 ± 0.17

4.2. Discussion

As it was stated by Abera (2003) and Oladeji *et al* (2016) this study also showed that, the knowledge of medicinal plant is still mostly handled by elders.(Abera, 2003; Oladeji, 2016). For current study three medicinal plants; *Croton macrostachyus, Calpurnea aurea and Vernonia myriantha* were selected for the antimicrobial activity test (Bisi-Johnson MA, Obi CL, Kambizi L, 2010; Yigezu *et al*., 2014). Additionally, *Zingiber officinale, Moringa oliefera* and *Allium sativum* were selected for its food preservative activity by considering its safety issue*(Padalia et al.*, 2016).

For current antimicrobial study test strains were isolated and identified by using morphological, endospore, shape and different biochemical tests as of it was identified by (Nemo *et al.*, 2017). Based on this *S. aureus* strain was identified by using mentioned techniques. Gram positive, catalase positive, cocci and non-spore forming isolate was taken and confirmed by using coagulase test. Then the coagulase positive isolate was taken as *Staphylococcus aureus* as it was also followed by (Gulani, 2016; Kaur *et al.*, 2018). Humam (2016), identified and characterized the strains of E. coli by using different morphological and biochemical tests. Following that, the colonies with gram negative, rod shaped, non-spore forming and catalase negative were selected and enriched in nutrient broth (Humam, 2016; Islam *et al.*, 2014). After enrichment for 24 hr, the isolates transferred to MacKonkey Media the selective media of *Eschercheria coli*. After growing them on on MacKonkey Agar Media all biochemical tests and morphological characterization suggested by (Al-baer *et al.*, 2017) were done including lactose fermentation and production of pink colonies on MacKonkey agar (Paramesh *et al.*, 2018). Additionally the strain of *B. cereus was* also isolated and identified. *Solani et al* (2019) isolated and identified B. cereus. In this study the isolate *B. cereus*, gram positive, catalase positive, rod, citrate positive, endospore positive, lecithinase test positive and motile strain was also confirmed and taken as *B. cereus* (Anonymous, 2018; Solanki *et al.*, 2019). Isolation and identification of *S. Typhi* was conducted. Rod-shaped Gram negative, non-lactose fermenting, non-sporing as it was used by (Oluyege *et al.*, 2015) presumed as Salmonella spp. Then colonies with black centers on Xylose Lysine Desoxycholate Agar (XLD) (Salm-surv *et al.*, 2015) were selected and different biochemical and morphological identifications were conducted as followed by (Oluyege *et al.*, 2015; Salm-surv et al., 2015) and used for antimicrobial activity test.

Antimicrobial activity test was done by diffusion disc method that was used by (Solanki *et al*., 2019). During antimicrobial activity test the diffusion discs were sterilized. After confirming all test strains they were preserved and used for antimicrobial activity test by activating. Crude of all plants extract re-dissolved in DMSO and disc of diffusion were placed in the solution of crude extract for 30 minute. From all crude extracts, chloroform extracted *C. macrostachyus* crude showed broad-spectrum antimicrobial activities on both bacterial and fungal test strains. From selected bacterial test strains *S. aureus* showed high susceptibility toward *Croton macrostachyus* chloroform extracted crude. After testing for antimicrobial activities of all crude extracts, *Croton macrostachyus* chloroform extracted showed the highest diameter of inhibition zone 23.33 ± 3.30 mm on *S. aureus*. Additionally, chloroform extracted *Croton microstachyus* crude showed zone of inhibition with diameter of 12.33 ± 2.87 mm on B. cereus and 22.33 ± 1.70 mm on *C. albicans*. This prove that, the response from the respondents to use *Croton macrostachyus* to treat wounds and many skin related diseases. Next to that *Croton macrostachyus* acetone extracted formed zone of inhibition with diameter of 17.67 ± 2.05 mm on *S.aureus,* 19.67 ± 0.94 mm on *C. albicans*. Methanol extracted *Croton macrostachyus* crude formed zone of inhibition with diameter of 12.67 ± 2.05 mm on *S. aureus* and 22.00 ± 0.82 mm on *C.albicans*. From all *Croton macrostachyus* crudes the chloroform extracted showed how highest inhibition zone on all test strains, while methanol extracted crude was the second most on *C. albicans*. When compared with each other *Staphylococcus aureus* was very sensitive to *Croton macrostachyus* chloroform extracted than other crude extracts. Two standard strains, S. aureus (ATCC 25923) and *E. coli* (ATCC 25922) were activated. After activating the strains antimicrobial activity of some crudes were tested on the strains and the result showed relationship with the result of isolate based antimicrobial activity and that of standard strains.

The minimum inhibitory concentration of *Croton macrostachyus* chloroform extracted was 0.235 ± 0.021 g/ml which was the minimum crude concentration that able to inhibit growth of *Staphylococcus aureus*. After conducting phytochemical screening the *Croton macrostachyus* crudes alkaloids, flavonoids, saponin, phenols, tannins, terpenes and steroids were detected in the crude. The same with *Croton macrostachyus, in crude of* Calpurnia aurea alkaloids, flavonoids, saponin, phenols, tannins, terpenes and steroids were detected in the crude(Elisha *et al.*, 2017; Mulata *et al.*, 2015).

Ginger showed abity to inhibit growth of microbial load on meat in food preservative activity. The log of microbial load of fresh chopped meat used for the activity was 1.92 ± 0.29. After 24 hr. of waiting for the evaluation of food preservative activity the load of control become 6.08 ± 0.66 while the load of experimental was 2.49 ± 1.30. Therefore the ginger have significant food preservative activity. The difference between the load of control and experimental was significant. Following the ginger Moringa also showed good food preservative activity. The log of microbial load of fresh chopped meat used for the activity was 2.60 ± 0.67. After 24 hr. of waiting for the evaluation of food preservative activity the load of control become 3.54 ± 0.81 while the load of experimental was 2.62 ± 0.33. The difference between the load of control and experimental was also showed food preservative activity. Food preservative activity of garlic was also performed. The log of microbial load of fresh chopped meat used for the activity was 2.37 ± 0.25. After 24 hr. of waiting for the evaluation of food preservative activity the load of control become 3.60 ± 0.44 while the load of experimental was 2.72 ± 0.17. The difference between the load of control and experimental was significant(Fatoki *et al.*, 2013).

5. Conclusion and Recommendations

5.1. Conclusion

The study showed, that the knowledge of medicinal plant are mostly in hands of elders and needs further documentation to be transferred from generation to generation. Additionally most of the knowledge of medicinal plants are mostly commons in residents around rural area. In this study the targeted object was to select medicinal plant for testing its antimicrobial activity after prioritizing the plants based on the response of the practitioners. Based on the frequency from the respondents three plants were selected from top five for antimicrobial activity test and three others selected for their safety. These plants were *Croton macrostachyus*, *Calpurnia aurea* and *Vernonia myriantha* for antimicrobial, *Zingiber officinale, Moringa oliefera and Allium sativum* for food preservative activity of that plant. The extraction was conducted on three plants *Croton macrostachyus*, *Calpurnia aurea*. Chloroform, acetone and methanol were used in extraction. In extraction process 40 gram of every powder was weighed and soaked in 150 ml of solvent. After soaking it for 72 hr the liquid was filtered by using filter paper and rotary vapour was used to remove the solvents from the crude. After waiting till it dry, the crude was re-dissolved in DMSO and used for testing antimicrobial activity. Five different strains of microorganisms *S. aureus, E. coli, B. cereus, S. Typhi* and *C. albicans* were used as testing microorganisms. After evaluating the antimicrobial activities of the crude extracts of *Croton macrostachyus* showed good antimicrobial activity on *S. aureus* and *C. albicans*. Additionally, minimum inhibitory concentration of those crude were determined by serially decreasing the size of the crude. Phytochemical screening of the crude of *Croton macrostachyus* and *Calpurnea aurea* showed that presence of alkaloids, flavonoids, saponin, phenols, tannins, terpenes and steroids in the crude. In other case three different plants powders (Ginger, Mouringa and Garlic) were evaluated for their food preservative activity. Then the powder of the ginger, moringa and garlic showed good activity by inhibiting the growth microbes in preserved food while comparing with the control.

5.2. Recommendations

After conducting the study on antimicrobial and food preservative activity of medicinal plants in the study area, the researcher forwarded the following recommendations:

- Further toxicity test should be conducted on *Croton microstachiyus* to use it as alternative source of treatment for disease
- Since antimicrobial activity of crude extract of the same plants depends up on solvents scholars have to identify the difference
- Every concerned body have to work on further study on antimicrobial activity of the crude extract on different species of bacterial and fungal strains
- Every concerned body have to work on standardization of medicinal plants
- Researchers can work on detecting food pathogens in the food preservative activities conducted in this study.

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Annexes

Annex 1: Questionnaire Prepared for Data Collection on Socio-demographic Characteristics of the Study Participants and Frequency of use of Medicinal Plants in Seka Chokorsa District

Jimma University

College of Natural Science

School of Postgraduate Studies

Department of Biology

Questionnaire on Antimicrobial Activities and Food Preservatives Activities of Medicinal Plants in Jimma Zone; Seka Chokorsa District

Dear respondent,

The purpose of this study is to gather information on the practice of Seka Chekorsa District community on the usage of traditional medicinal plants used to treat human disease. Findings of this study will have significant importance in documenting and evaluation of antimicrobial activities of extracts from that plant. In addition the evaluation of food preservative activities will be took place by this study. Thus, feel free and respond to the questionnaire genuinely. Your answers will be kept confidential and used only for research purpose. You have the right to decline responding to the questions if you don't feel comfortable.

Thank you in advance for your time and kind cooperation.

I) **General information about the respondent**. Mark "X" on your appropriate choice.

1. Age: ☐ Less than 24
   ☐ 25-32
   ☐ 33-40
   ☐ Above

2. Sex: Male ☐ Female ☐

3. Practice of Medicinal Plants:
   ☐ Vendor of Traditional Medicinal Plants
   ☐ User

4. Level of Education:
   i. Illiterate ☐ ii. Literate ☐

5. Residence:
   Urban ☐
   Rural ☐

6. What is your monthly income?
   i. Less than 500 Birr. ☐
   ii. 500 – 1000 Birr. ☐
   iii. 1000 – 2500 Birr ☐
   iv. Above 2500 Birr. ☐

II) Foodborne disease: Mark "X" on your appropriate response.

6. Diarrhea:

   a. Dou you have any practice on traditional medicinal plant that can be used as diarrea cure? Yes ☐ ☐

   b. If "Yes" what is the local name of that plant?

      i. _____

   c. Which part of the plant is used as medicinal?

      Leaf ☐ Stem ☐ Root ☐

      Bark ☐ Other ☐ _____

   d. If there is any comment

      i. _____

III) Skin diseases

7. Wound

   a. Dou you have any practice on traditional medicinal plant that can be used as wound cure? Yes ☐ No ☐

   b. If "Yes" what is the local name of that plant?

      ii. _____

   c. Which part of the plant is used as medicinal?

      Leaf ☐ Stem ☐ Root ☐

      Bark ☐ Other ☐ _____

   d. If there is any comment

      iii. _____

8. Fungal skin disease

   a. Dou you have any practice on traditional medicinal plant that can be used as fungal skin disease cure? Yes ☐ No ☐

   b. If "Yes" what is the local name of that plant?

      iv. _____

   c. Which part of the plant is used as medicinal?

      Leaf ☐ Stem ☐ Root ☐

      Bark ☐ Other ☐ _____

   d. If there is any comment

      v. _____

9. Other skin disease you know and its cure:

   a. Dou you have any practice on traditional medicinal plant that can be used as other skin disease cure? Yes ☐ ☐

   b. If "Yes" what is the local name of that plant?

      vi. _____

   c. Which part of the plant is used as medicinal?

      Leaf ☐ Stem ☐ Root ☐

      Bark ☐ Other ☐ _____

   d. If there is any comment

      vii. _____

Annex 2: Result of some biochemical tests

Annex 3: Result of Biochemical and shape characterization

No.	Suspected strain	Gram staining	Endospore test	Shape of bacterial cell	Citrate utilization test	Urease test	H_2S formation	Indole test	Gelatin hydrolyses test	Motility test	Catalase test
1.	*Eschercheria coli*	-	-	Rod	-	-	-	+	-	+	+
2.	*Bacillus cereus*	+	+	Rod		NA	NA	-	-	-	+
3.	*Staphylococcus aureus*	+	-	Cocci		+	-	-	+	+	+
4.	*Salmonella typhi*	-	-	Rod		-	+	-	-	+	+
5.	*Candida albicans*	Available strain in Research and post graduate Laboratory, Jimma, Jimma University									

[i] **Madhanialem Restaurant** is located in Ginjo Guduru kebele, Jimma city, Oromia, Southwest Ethiopia

[ii] **Meat firfir** is a combination of shredded injera, meat, berbere, onions, and clarified butter.