JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF CHEMISTRY

M.Sc THESIS

ON

PHYTOCHEMICAL INVESTIGATION OF ROOT BARKS OF*Securidaca longipedunculata* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

BY

TAHIR TIKISA

OCTOBER, 2017

JIMMA, ETHIOPIA

PHYTOCHEMICAL INVESTIGATION OF ROOT BARKS OF*Securidaca longipedunculata* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

BY

TAHIR TIKISA

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ORGANIC CHEMISTRY

ADVISORS

NEGERA ABDISSA (PhD)

DELE ABDISSA (M.Sc)

OCTOBER, 2017

JIMMA, ETHIOPIA

A Thesis submitted to School of Graduate Studies Jimma University in partial fulfillment of the requirements for the degree of Master of Science in Organic Chemistry

Advisor Signature Date

Negera Abdissa(PhD) _____ _____

Department of Chemistry

College of Natural Sciences

Jimma University

Co-Advisor

Dele Abdissa (M.Sc) _____ _____

Department of Chemistry

College of Natural Sciences

Jimma University

DECLARATION

I, the undersigned affirm that this thesis is my original work and has not been presented for research in any other university.

_____

Tahir Tiksa

This M.Sc Thesis has been submitted with our approval as supervisors:

_____

Negera Abdissa(PhD)

Chemistry Department

Jimma University, Ethiopia

_____

Dele Abdissa (M.Sc)

Chemistry Department

Jimma University, Ethiopia

Contents

page

List of Figures

List of Tables

Table page

Acknowledgement

First I would like to thank the highest merciful Allah for giving me the strength and the calm throughout my study.

I would like to express my sincere and heartfelt gratitude to my advisors Dr. NegeraAbdissaand Mr. Dele Abdissa for their continuous, unreserved, constructive guidance and support as well as providing the necessary resources unlimitedly. I am very fortunate and proud to have worked under their guidance.Ms.BurtukanHamba is also thanked for her continual encouragement, advice and financial support. My gratefulness goes to BESS, for giving me the opportunity to study and for providing me financial support for thesis research work.I would like to extend my gratefulness to Jimma University College of Natural Science, Department of chemistryfor providing me with chemical and material support. Equally I would like to thank Biology Department for their assistance on antibacterial assay.I wish to sincerely acknowladged the International Foundation for Science(IFS, Grant NO: F/5778-1) for chemical support for isolation of the compounds.I would like to express my gratitude to JemalSuleman and BulchaTarressafor informing me the places where the plants arefound.

Abstract

The discovery of new antimicrobial compounds from medicinal plants mainly from those which have documented traditional uses is encouraging and believed to solve these microbial burdens. Therefore, the aim of this study was to isolate and characterize antimicrobial compounds from the root bark of *Securidaca longipedunculata*, aplant commonly used for the treatment of sexually transmitted disease, fungal infection, and wounds in Ethiopia. The dried and powdered root bark was extracted with increasing gradient solvent (hexane, chloroform, acetone and methanol) about 5 g (0.53%), 12.5 (1.32%), 10 (1.05%), 40 g (4.23%) of crude extract of n-hexane, chloroform, acetone, and methanol, respectively were obtainedand screened for antibacterial activity on four pathogenic bacterial strain and phytochemical analysis was carried out to determine the secondary metabolites in each extract.Phytochemical screening revealed that terpenoids, coumarins and Saponins are present in the entire crude extracts, alkaloids and quinone were present in acetone and methanol extract only, and phenols and flavonoids were present in all the extract except hexane extract. The acetone extract was subjected to column chromatography for further purification using ethyl acetate and n-hexane.The first fraction afforded 12 mg of compound **1**, the second fraction afforded 13 mg compound **2** and the third fraction afforded 20 mg compound **3.**The isolated compounds were characterized by the various spectral techniques: IR, UV, ^{1}H NMR, ^{13}C NMR, DEPT-135, HMBC and HSQC. The antibacterial analysis showed that the acetone extract was the most active with the mean zone of inhibition30mm followed by methanol extract 28 mm, chloroform 16 mm and hexane extract 8 mm respectively. Compound **3**showed moderate antibacterial activity with 12 mm zone of inhibition.These results verified the claims by traditional healers inEthiopia to treatedifferent disease using this plant.

Abbreviations

WHO	World Health Organization
CC	Column Chromatography
TLC	Thin Layer Chromatography
DMSO	Dimethyl Sulfoxide
NMR	Nuclear Magnetic Resonance
1D NMR	One Dimensional Magnetic Resonance
2D NMR	Two Dimensional Magnetic Resonance
DEPT	Distortion less Enhancement by Polarization Transfer
IR	Infra-Red
UV	Ultraviolet Visible
MIC	Minimum Inhibition Concentration
SL	*Securidaca longipedunculata*
HMBC	Hetero Nuclear Singular Quantum Correlation
HMQC	Hetero Nuclear Multiple Quantum Correlation

1. Introduction

1.1 Background of the Study

Since ancient times, plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock. The application of medicinal plants is almost as old as the history of mankind. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 B.C. in China, and 1600 B.C. by Syrians, Babylonians, Hebrews and Egyptians. Considerable indigenous knowledge, from the earliest times, is linked to the use of traditional medicine in different countries [1].

The use of traditional medicine and medicinal plants in third world countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutic from these plants as well as from traditionally used rural herbal remedies [2, 3].

Ethiopia has a long history of traditional medicines and has developed ways to combat disease utilizing them. The ways are also as diverse as the different cultures, language and belief**.** In fact, in Ethiopia up to 80% of the population use traditional medicine due to the cultural acceptability of healers and local pharmacopeias, relatively low cost of traditional medicine, difficult access to modern health facilities and due to various side effects caused by modern synthetic medicines [4].

Healing of traditional medicine in Ethiopia is not only concerned with curing of diseases but also with the protection and promotion of human physical, spiritual, social, mental and material wellbeing. It is widely believed in Ethiopia that the skill of traditional health practitioners is 'given by God' and knowledge on traditional medicines is passed over orally from father to a favorite child, usually a son or is acquired by some spiritual procedures [5].

Plants produce an enormous variety of natural products with highly diverse structures. These products are commonly termed as primary metabolites and secondary metabolites. Primary metabolites are responsible for the growth and development of plants, for example; sugars, protein, lipids, starch. Secondary metabolites appear to function primarily in defense against predators andpathogens and in providing reproductive advantage. They may also act to create competitive advantage as poisons of rival species. Some of the most important secondary metabolites (bioactive phytochemical) constituents are alkaloids, essential oils, terpenoids, Saponins, flavonoids, tannins and phenolic compounds [6- 8].

These secondary metabolites are plant-derived substances that have a great interest owing to their many different functions. Medicinal plants are the richest bioactive resource on drugs of traditional systems of medicine, modern medicines, and chemical entities for synthetic drugs. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, in particular the synergistic effects and polypharmacological application of the plant extracts [9 -11]

The World Health Organization (WHO) estimated about 80% of the people in the developing countries relies on traditional medicine for primary health care needs, and most of this therapy involves the use of plant extracts or their active component [12]. Herbal remedies have been used for centuries but more recently, the compounds that are active have been identified and synthetic organic chemists have then been able to produce the molecules on large scale along with their synthetic analogues [13].

In fact plants generate a diverse range of biologically active molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a crucial role in the maintenance of human health since ancient times. More than 50% of all modern clinical drugs are of natural product origin and natural products play a dominant role in drug development programs in the pharmaceutical industry [14].

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom has been a major driving force in the field of natural product research. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Nowadays multiple drug resistance has developed due to the misuse of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents.

Hence, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [14]

1.2 Statement of the problem

The emergence of various drug-resistant organisms limited therapeutic efficacy of many of currently available antimicrobial drugs. The search for structurally varied compounds from medicinal plants mainly from those which have documented traditional uses should be over emphasized.It has been claimed that about 80% of the Ethiopian population rely on medicinal plants for treating various illnesses [4]. However, there is a need for scientific support for plants used in the traditional medicine before these could be recommended for treatment of illness. Medicinal plants have a promising future, because there are about half million plants around the world, and most of their medical activities are not investigated yet, that their medical activities could be decisive in the treatment of present or future studies.

In line with this fact, it is important to note that a common practice in, Oromia region , Sayo district, where the root bark of *Securidaca longipedunculata (shote in Afaan Oromo, Etsmenay in Amharic)*istraditionally used by the community for the treatment of sexually transmitted disease, malaria, fungal infection, diabetes, and wounds. The genus *Securidaca*belongs to the family Polygalaceaewhich have been known to contain a large number of chemically complex bioactive compounds such as flavonoids, terpenes, coumarins and steroids.However, the phytochemicals information and bioactivity of the molecules from the root bark of *Securidaca longipedunculata*, which has been widely practiced by the local community for its medicinal role has not been sufficiently reported so farby many researchers in Ethiopia.Therefore this research will provide promising information of the presence of chemicals that can be used as a medicinal value, evaluation of its antibacterial activities and motivates for further advanced researchers.

1.3 Objectives

1.3. 1 General objective

- The main objective of this study was to carry outphytochemical investigation of root barks of *S. longipedunculata* and evaluation of its antibacterial activities.

1.3.2 Specific Objectives

- To isolate compounds from the root barks of *S. longipedunculata* using chromatographic technique;
- To characterize the structure of the isolated compounds using spectroscopic techniques including UV, IR, 1D and 2D NMR.
- To evaluate antibacterial activity of the crude extracts and isolated compounds on the four bacterial strains such as*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and*Bacillus subtilus*.

1.4 Significance of the Study

The findings of this research would:

- ❖ Help to provide the chemical profile of root bark of *S. longipedunculata*.
- ❖ Identify the antibacterial activity of the phytochemicals from root bark of *S. longipedunculata.*
- ❖ Put base line information for the further studies on the same plant genus.

2. Review of Related Literature

2. 1. Botanical information

The genus *Securidaca* comprises about 80 species, characterized by papillionaceous purplish flowers and mostly scandent shrubs and lianas [15, 16].*Securidaca longipedunculata* Fresen, (synonyms *Securidaca longipedunculata* var. *longipedunculata* or *Elsota longipedunculata*, family Polygalaceae) is a small tree up to 6 meters high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves that are variable in size and shape and crowded towards the stem tips [17]. Clustered flowers are small, pink to lilac or purple in colour, sweet scented and are produced in early summer [18]. Fruits are a round nut, heavily veined, occasionally smooth, oblong, purplish green when young and possess a membranous wing of about 4 cm long [19].

Securidaca longipedunculata is grown in a wide range of climates, from subtropical, hot and arid climate to summer rainfall and equatorial humid. It occurs in a broad range of vegetation, from semi-arid scrub to dense forest, including many woodland and bush habitats and gallery forests. S. longipedunculata is resistant to bush fires and is frost sensitive [20].It is widely used medicinal plant in African countries, including Angola, Benin, Botswana, Burundi, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe, Mozambique, as well as in the North West and Limpopo Provinces of South Africa[21, 22].

Figure 1.*Securidaca longipedunculata* tree (picture taken by author)

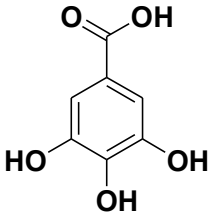
2.2 Ethnomedicinal uses

The major Ethnomedicinal uses of roots of *S. longipedunculata* in different countries are venereal diseases, syphilis, pains, fever, epilepsy, pneumonia, tuberculosis, malaria, ascariasis, abortion, constipation, coughs, sexual boost, toothache, rheumatism, as an aphrodisiac and tick prevention in animals [23-26]; the Leaves used for dislocated jaw, headaches, skin cancer, skin infections and contraceptive purposes [27, 28]. The stem bark used to treat infections related to nervous and circulatory system, dysentery, malaria, typhoid and frequent stomach ache [29, 30]. These suggest that the most commonly used plant part is the root and that the species is used in the treatment of a variety of ailments including coughs, fever, malaria, tuberculosis and sexually transmitted diseases indifferent geographical areas. This provides support for a pharmacological basis of the use of the plant species in the treatment of such ailments.

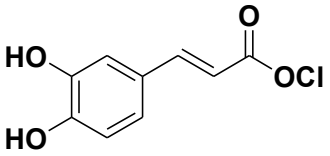
2.3 Phytochemistry of the species *S. longipedunculata*

The water and aqueous methanol extracts from the root yielded a variety of compounds in varying amounts, including gallic acid (**1**), chlorogenic acid (**2**), p-coumaric acid (**3**), caffeic acid (**4**), cinnamic acid (**5**), epicatechin (**6**), apigenin (**7**), and quercetin dihydrate (**8**) [31].

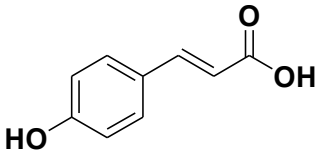
The aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, Saponins, tannins, volatile oils, terpenoids and some steroids [32-34] while chloroform and ethanol extracts indicated flavonoids, Saponins, coumarins, tannins and alkaloids [35].
1, 7-dihydroxy-4-methoxyxanthone **(9**), benzyl -2-hydroxy-6-methoxy benzoate (**10**), methyl-2-hydroxy-6-methoxy benzoate (**11**) were isolated from the root bark of dichloromethane extract of *S. longipedunculata.*Whereasthe 1, 3, 6, 8- tetrahydroxy-2, 5-dimethoxyxanthone (**12**) and 1, 6, 8-trihydroxy-2, 3, 4, 7-tetramethoxyxanthone (**13**)[36] were also isolated from the acetone extract of the fresh root bark. Moreover, the hexane extract of the root indicated the presence of 1, 5-dihydroxy-2, 3, 6, 7, 8-pentamethoxyxanthone (**14**), 2-hydroxy-l, 7-dimethoxy xanthone (**15**) and 1, 6-dihydroxy-xanthone (**16**) [37] are some of the compounds isolated from *S. longipedunculata* are shown in figure 2.


O
OH
HO
OH
OH

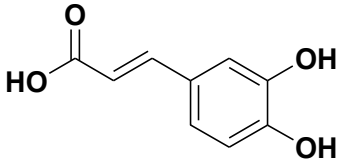
1


HO
O
OCl
HO

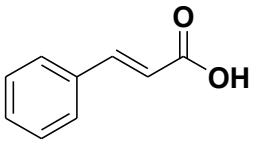
2


O
OH
HO

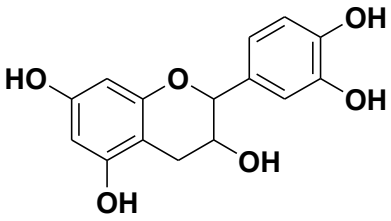
3


O
HO
OH
OH

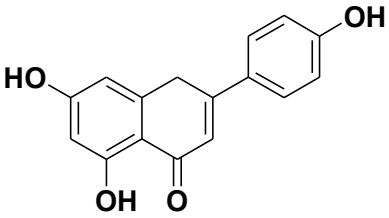
4


O
OH

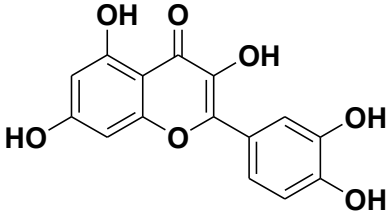
5


OH
HO
O
OH
OH
OH

6

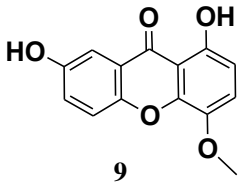

OH
HO
OH
O

7

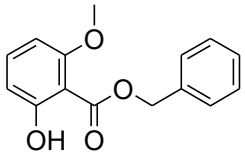

OH
O
OH
HO
O
OH
OH

8

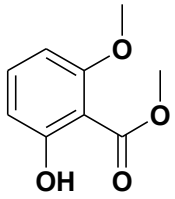
,



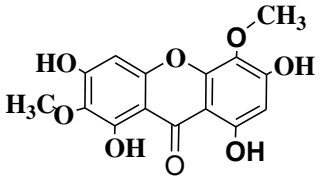
9



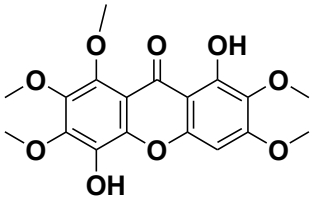
10



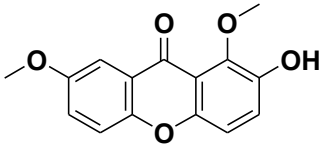
11



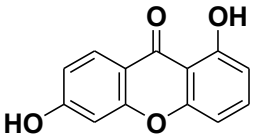
12



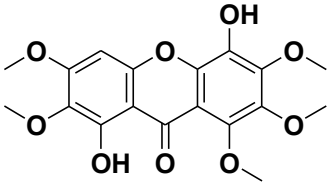
13



14



15



16

Figure 2. Compounds Repoeted from the plant

2.4 Earlier studies done on medicinal value of *Securidaca Longipedunculata*

Extracts of the stem bark and roots of *Securidaca longepedunculata* are used in traditional medicine for the treatment of bacterial infections [38-41].In a disc diffusion study, the aqueous leaf extract yielded inhibition zones of 15 mm against both *E. coli* and *S. typhi*, while the chloroform leaf extracts exhibitedinhibition zones of18 mm against *P. aeruginosa* at a concentration of 7.5 mg/disc[42].

The methanol extracts and the chloroform fraction of the root bark hasinhibition zone of 28 mm against methicillin resistant Staphylococcus aureus, while hexane and ethyl acetate fractions exhibited inhibition zone ranging from 14 to 19 mm against *S. pyogenes, P. fluorescens* and *Klebsiella pneumonia*[43]. Theethanol extracts of the root bark at a concentration of 100 mg/mlhasinhibitionzones of 15.10 mm [44].

In the broth micro dilution assay, the chloroform extracts of the leaf had a minimum inhibitory concentration (MIC) of 0.591 mg/ml against both *S. typhi* and *P.aeruginosa* while the aqueous extracts of the leaf revealed MIC of 6.25 mg/ml and minimum bactericidal concentration (MBC) of 62.5 mg/ml against *S. typhi*. Besides exhibiting a MIC of 0.313 and MBC of 0.625 mg/ml against both *S. aureus* and *P.aeruginosa*t[45]suggesting that the extract may be a good source of antibacterial compounds. The extract of the acetone soluble portion of the root exhibited a potent MIC of 0.02 mg/ml against *B. subtilis* and *S. typhi* [46]. Moreover, similar extract exhibited MIC of 0.10 mg/ml against both *E. coli* and *P. aeruginosa*. In other reports, the essential oil from *S. longipedunculata* had MIC of 12.79 mg/ml against *E. coli* [47] while the 70% methanol extract of the leaves exhibited a MIC of 0.45 mg/ml and 0.23 mg/ml against *S. marcescens* and *S. flexneri* respectively [48].

The dichloromethane extract of the leaves showed anti-plasmodial activity with an IC_{50} of 6.9 µg/ml against *Plasmodium falciparum*[49], while the methanol extract of the root suppressed *Plasmodium berghei* by 82% at a dose of 0.56 mg/kg [50]. Furthermore, the methanol and chloroform extracts of the root exhibited an IC_{50} of >250 µg/ml against the chloroquinone resistant *P. falciparum* strain[51].In other reports, extracts from seeds of *S. longipedunculata* did not show any activity at 50 µg/ml against *P. falciparum* FCA-2 [52], suggesting that the antimalarial compounds may only be present in the leaves and roots.

3. Material and Methods

3. 1 Chemicals and Material

Chemicals used were analytical reagent grade n-hexane, chloroform, acetone and methanol solvents were used for successive extraction and ethyl acetate in n-hexane is used for column elution. Columnchromatography was performed on oxalic acid impregnated silica gel (the silica gel was deactivated by mixing 1Kg silica gel (60-120 mesh) with 3% of oxalic acid (30 g in 1L of distilled water). And allowed to stand for 30 min, filtered and dried in an oven at 100^{o}C for 45 min).Standard antibiotic drug (gentamicine 5 µL), Mueller Hinton agar, nutrient agar and saline solution were used as a culture medium during antibacterial test.

Thin layer chromatography (TLC) was performed on pre-coated silica gel on aluminum foil and viewed under UVchamber (LF-260.LS,EEC) (254 and 365 nm) for detection of spot. The material used were rotary evaporator (Heidolph Laborata 4000), UV/VIS(JENWAY6705,UK), Infrared (IR) spectra were measured on Perkin-Elmer IR spectrophotometer. The ^{1}H-NMR, ^{13}C-NMR and DEPT-135 and 2D spectra were recorded on Bruker Avance NMR in acetone (D_6) solvent.

3.2 Plant material collection and preparation

The root bark of *S. longipedunculata* was collected from Western Ethiopia, Oromia regional State, Kelem Wollega Zone, Sayo district about 636 km away from Addis Ababa in March 2017. The collected root bark was washed by running water, air dried, and powdered by using pestle and mortar and the powder was stored in appropriate container until used for extraction.

3.3 Extraction

About 945 gm of the air dried and powdered sample was sequentially extracted with an equal volume of about 2.5 L, hexane, chloroform, acetone and methanol using cold maceration technique for 72 hr. at room temperature. The crude extracts of each solvent were filtered first through a fresh cotton plug and then through Whatman (No.1) filter paper. The filtrates were concentrated by evaporation under reduced pressure using a rotary evaporator at 40 °C and about 5 g (0.53%), 12.5 (1.32%), 10 (1.05%), 40 g (4.23%) of crude extract of n-hexane, chloroform, acetone, and methanol, respectively were obtained. All the extracts were stored in the desiccators until used for isolation and microbial assay.

$$yield = \frac{weight\ of\ the\ crude\ extract\ (g)}{weight\ of\ the\ draied\ sample\ usd\ (g)}\ x\ 100$$

3.4Phytochemical screening

The crude extract of n-hexane,chloroform, acetone and methanol of the plant were subjected to qualitative chemical screeningfor the identification of the various class of active chemical constituent (Flavonoids, Alkaloids, Saponins Terpenoids, Tannins coumarins, Quinone and Phenols) in securidica longipendunculata root bark using the method described by Sofowora and Harborne methods[53, 54].

Flavonoids: were identified by adding 5 mL of dilute ammonia solution to an aqueous filtrate of the extract, followed by the addition of concentrated sulphuric acid. A development of yellow coloration indicate the presence of flavonoids.

Alkaloids: were tested by adding a few drops of acetic acid to 5ml of an extract followed by modified Dragendroff's reagent (potassium iodide mixed with bismuth oxynitrate) and mixed well. Orange red precipitate formation indicate the presence of alkaloids.

Saponins: were tested by boiling 2 grams of the powdered sample in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate mixed with 5ml of distilled water and was shaken vigorously for a suitable persistent froth. The frothing mixed with 3 drops of olive oil and shaken vigorously, and formation of emulsion observed indicated the presence of Saponins.

Terpenoids: detection was carried out by mixing 5ml of plant extract with 2ml of chloroform and concentrated sulphuric acid added to form a layer. A reddish brown coloration of the interface formed show presence of Terpenoids.

Tannins: were identified by boiling 0.5gram of the powdered sample in 20ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride added and observed for brownish green or a blue-black coloration indicatpresence of tannins.

Phenols: were identified by adding few drops of drops of methanol and ferric chloride solution to the plant extract mixed. A blue green or red colour development indicate the presence of phenol.

3.5 Isolation of compounds

The appropriate solvent system for isolation of compounds was made after carrying out the TLC analysis of the crude extract in various combinations of solvents with increasing polarity. Hence, n-hexane in ethyl acetate was found to show good separation. Then, the column was packed with deactivated 120 g on oxalic acid impregnated silica gel. Then, the acetone crude extract was subjected to chromatographic separation. And about 15 g was adsorbed.

3.6 Evaluation of antibacterial activity of crude extracts and isolated compound

The crude extracts and isolated compound were subjected to antibacterial test.The test solution of the extracts were prepared by dissolving 200 mg of crude extract in 1 mL of dimethyl sulfoxide (DMSO) to get the final stock concentration of 200 mg/mL solution of the test sample. For compound **3**,12.5 mg in 0.25 mL of DMSO to get 50mg/mL concentration. The bacterial strains used were Gram positive bacteria *Bacillus subtilus* and*Staphylococcus aureus*andGram negative *Escherichia coli* and *Pseudomonas aeruginosa.* These standard bacterial were obtained from the Department of Biology, Jimma University.

The antibacterial activity test was done using disc diffusion method following a standard procedures [55]. Bacterial strains were grown and maintained on an agar slant at controlled temperature (4°C). The strains were activated for experiment at 37°C for 24 hours on nutrient agar, before any experimental screening. The culture media was boiled in distilled water to dissolve the media and autoclaved at 121^{o}C for 2 hr. and poured into sterile Petri dishes and after solidification it was placed in refrigerator until use.

Four well-isolated colonies of bacteria were selected from an agar plate culture and the top of each colony was touched with a loop, and the growth was transferred into a tube containing 4.5ml of a suitable nutrient broth medium. The broth culture was incubated at 37^{O}C until it achieves or exceeds the turbidity of the 0.5 McFarland standards for 8 hours.

The turbidity of the actively growing broth culture was adjusted with sterile saline solution to obtain turbidity optically comparable to that of the 0.5 McFarland standards which was resulted in a suspension containing approximately 1 to $2x10^8$CFU/mL for different strains. Inoculums containing 1 - 2 x 10^8CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. 200mg/mL and 50 mg/mL concentration of 20µL of the working suspension of the same concentration of the sample plant extracts, isolated compound and the same volume DMSO for negative control was impregnated using Whatman No.1 filter paper disc (diameter 6mm) with the help of micropipette respectively. Positive control using Gentamicin was assayed simultaneously. Plates were left for 10 minutes till the extract diffuse in the medium with the lid closed and incubated at 37°C for 24 h. After incubation, the plates were observed for the zone of inhibition and the diameter of the inhibition zone was measured using transparent ruler and mean was recorded.

4. Result and Discussion

4.1 Extraction Yield

Extraction yield is the ratio of mass of crude extract to mass of drained sample multiplied by 100. As the polarity of extracting solvent increase the mass (percentage yield) of the extract increase. Thus by the principle like dissolves like non polar and less polar substance are soluble in nonpolar and moderate polar solvent and polar substance are soluble in a polar solvent. As the polarity of the extract solvent increase gradually the selectivity of the solvent toward the solute increase. This result showed that most secondary metabolites in a *S. longepedunculata* root bark are polar.Percentages yield of crude extract were calculated by following the formula;

$$\%\ \boldsymbol{yield} = \frac{\boldsymbol{weight\ of\ the\ crude\ extract\ (g)}}{\boldsymbol{weight\ of\ the\ draied\ sample\ usd\ (g)}}\ \boldsymbol{x\ 100}$$

Table 1. Percentage yield of extracts of each solvent.

Solvent type	Mass (g)	% yield
n-hexane	5	0.53
chloroform	12.5	1.32
acetone	25	2.65
methanol	40	4.23

4.2 Phytochemical screening result

Qualitative phytochemical analyses for alkaloids, phenols, saponins, terpenoids, flavonoids quinone and coumarins were screened in all extracts of the root bark of *Securidaca longipedunculata*. The results of screening of the extracts indicated the presence of alkaloids, phenols, Saponins, terpenoids, flavonoids, Quinone and coumarins in the root bark extracts of the plant (Table. 2).

Table 2. Phytochemical screening of *Securidaca longipedunculata*root bark extract**.**

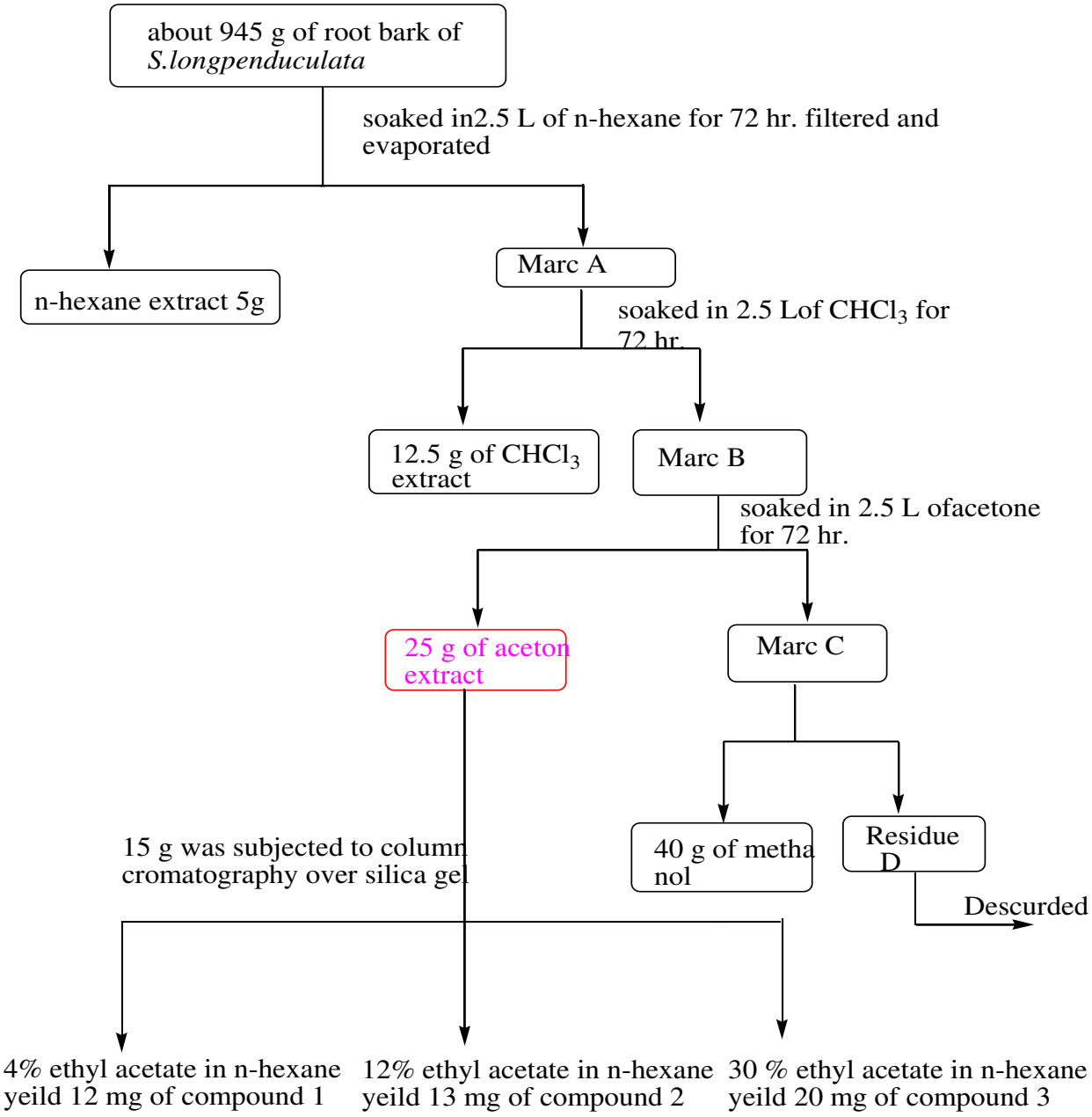
Constituents	Crude extracts			
	n-hexane	chloroform	acetone	Methanol
Alkaloid	_	_	+	+
Phenol	_	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	++	++
Terpenoids	+	+	+	+
Coumurins	+	+	+	+
Quinone	_	_	+	+

Key: + = the presence, - = absence and ++ = present in high concentration

Quinone and alkaloids were detected in acetone and methanol extract only. Saponins are present in all extracts, but the higher quantities of saponins were detected in methanol and acetone extracts. Phenols and flavonoids were present in the entire extracts with the exception of hexane extract. Terpenoids and coumarins were detected in all extracts. This confirms the presence of the Phytoconstituents responsible for antimicrobial activity in the root bark extracts.

4.3 Results of extraction and Isolated compounds

Hexane, chloroform, acetone and methanol extract produce 5, 12.5, 25 and 40 g respectively. Acetone extract was adsorbed on imprigenated silica gel,It was first eluted with 100% n-hexane, and then with increasing gradient of ethyl acetate in n-hexane (2, 4, 6, 8, 10, 12, 14, 16 and up to 50%) was used as eluent during separation. A total of 210 fractions each with 30 mL were collected. The fractions were concentrated under reduced pressure using a rotary evaporator at 40°C. The identities of the fractions were examined by TLC. The spots developed were visualized under UV light at 254 and 365 nm and then by exposure to iodine vapor. The fractions that showed the same TLC profiles (*Rf* value) were combined and concentrated.Among the fractions, 42-56 (4% ethyl acetate in n-hexane) were combined and further purified to give 12 mg of white amorphous solid compound labeled as compound **1**. Fractions 99- 101 (12% ethyl acetate in n-hexane) combined to give 13 mg of dark yellow compound labeled as compound **2**. Fractions 147-162(30% ethyl acetate in n-hexane) were combined to give 20 mg of yellow compound labeled as compound **3**.The isolated compounds were characterized by the various spectral techniques: UV IR, 1D (^{1}H-NMR, ^{13}C-NMR and DEPT-135) and 2D (HSQC and HMBC)


about 945 g of root bark of *S.longpenduculata*
soaked in2.5 L of n-hexane for 72 hr. filtered and evaporated
n-hexane extract 5g
Marc A
soaked in 2.5 Lof $CHCl_3$ for 72 hr.
12.5 g of $CHCl_3$ extract
Marc B
soaked in 2.5 L ofacetone for 72 hr.
25 g of aceton extract
Marc C
15 g was subjected to column cromatography over silica gel
40 g of metha nol
Residue D
Descurded
4% ethyl acetate in n-hexane yeild 12 mg of compound 1
12% ethyl acetate in n-hexane yeild 13 mg of compound 2
30 % ethyl acetate in n-hexane yeild 20 mg of compound 3

Schemfor the general procedure of extraction and isolation of compounds from the root of securidicalongipendunculata

4.4 Structural elucidation of the Isolated Compounds

Characterization of compound 1

Compound **1** was isolated as white amorphous solid. Its IR spectral analysis (appendix**1A**), showed a strong band in the region of 3238and 2917 cm^{-1} for the hydroxyl (OH) stretching and aromatic (C-H) stretching vibrations respectively. Other characteristic observation for aromatic C=C stretching, carbonyl (C=O) bond vibrations and C-O bond was also observed at 1445, 1659 and 1249 cm^{-1} respectively.

^{1}H-NMR (acetone-d_6 400 MHz) of compound **1**(Appendix**1B)** showed signals at 8.34 and 11.05 δ ppm for the hydroxyl on aromatic ring and hydroxyl on carboxylic acid respectively. Also four proton spin systems at δ 6.94, 7.52, 7.55, and 7.99 ppmwere observed (Table 3).The ^{13}C-NMR spectra (Appendix**1C**) of compound **1** showed 7C signals, comprising 6 aromatic C and one carboxylic C.The DEPT-135 spectra (Appendix**1D**) showed four methine group and three quaternary C. The aromatic carbons showed signals at δ 113.2, 118.1, 119.9, 131.2, 136.7 and 163.0ppm, the carbonyl C resonated at δ 172.7ppm (Table 3).Based on the above spectroscopic data the compound was identified to be 3-hydroxy benzoic acid (**1**) (Figure 3)

Table 3. ^{1}H NMR and ^{13}C NMR data of compound **1**

Position	**δ ^{1}H, *J*in HZ**	**δ ^{13}C**
1	—	136.7
2	7.55 *dd* (J = 8, 4)	113.2
3	—	163.0
4	6.94*dd (J= 16, 8))*	118.1
5	*6.96 m, (J = 8)*	131.2
6	7.62 *dd*(*J* = 8, 4)	119.9
7	—	172.7
3 OH	8.34	—
7 OH	11.05	—

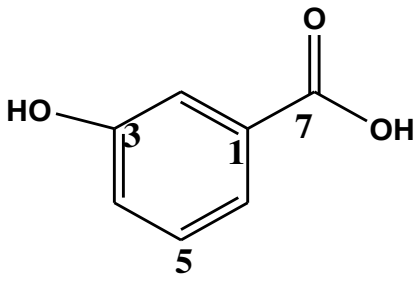


Figure 3 . The structure of compound **1**

Characterization of compound 2

Compound **2**was isolated as dark yellow solid. ^{1}H-NMR (acetone-d_6 500 MHz) spectrum of compound **2**(Appendix **2 A)** showed signals at δ 7.58(1H, *d, = 5*), 6.2 (1H, *dd, J*= 10 Hz) and 6.93 (1H, *d, J*= 5 Hz) for the protons on aromatic ring and 3.92 (3H, s) ppm for methoxylprotone. Protons of hydroxyl and carboxylic acids are not observed in 1D and 2D spectra, because they are exchangeable protons. The signals of exchangeable protons may be broad, difficult to see and move chemical shift position depending on the sample concentration and temperature.

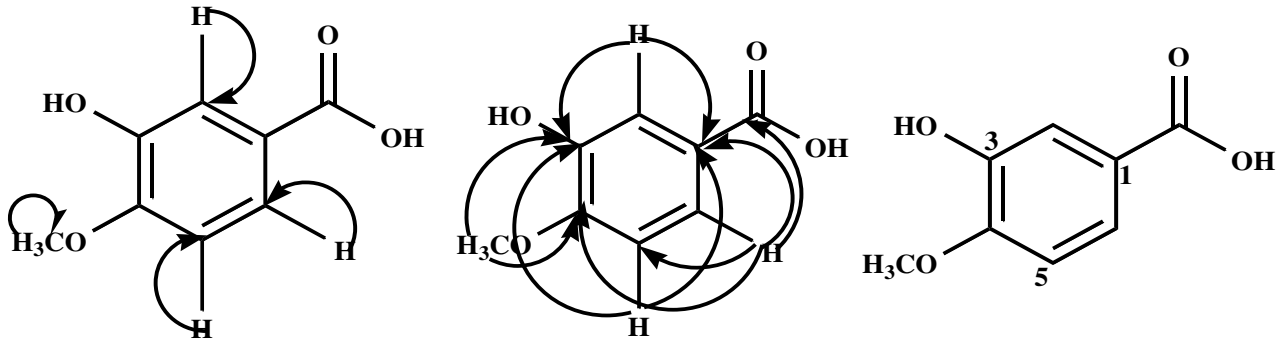
The ^{13}C-NMR spectra (Appendix**2B**) of compound **2**showed 8 C signals, comprising 6 aromatic C, one methoxyl C and one carboxylic C.The aromatic carbons showed signals at δ 122.0, 112.5, 147.1, 151.1, 114.6 and 124.0 ppmfor C 1 to C 6 respectively, the methoxy and carboxylic C resonated at δ 166.52 and 55.4 ppm respectively (Table 4).The assignment of protons, methoxy and quaternary carbons were assigned from hetro nuclear singular quantum correlation (HSQC) and hetero nuclear multiple quantum correlation (HMBC) spectra (Appendix **2C** and Table. 4). HSQC spectra of compound **2** showed the three aromatic protons were connected to C 2, C 5 and C 6 (Figure 4).

The position of the protons on aromatic ring and methoxyl were farther confirmed by HMBC. The connection of the methoxyl moiety at C-4 was established on the basis of the HMBC correlation of H-on methoxyl to C 3 and C 4.Three aromatic protons were assigned to position C-2, C-5and C-6, from the HMBC correlations of H-2at δ7.58 (1H, d) with C-1, (δ C 121.98), C2 (δ C 112.53), C3 (δ C 147.14), C4 (δ C 151.13),C6 (δ C 123.95) andC 7, (δ C 166.52) ; and H-5at δ6.93 (1H, d) with C 1, (δ C 121.98), C3 (δ C 147.14), C4 (δ C 151.13) andC6 (δ C 123.95) and H-6at δ7.62 (1H, dd) with C 1, (δ C 121.98), C2 (δ C 112.53), C4 (δ C 151.13),C6 (δ C

123.95) and C 7, (δ C 166.52) ppm. Based on the above spectroscopic data the compound was identified to be 3-hydroxy- 4-methoxy benzoic acid (**2**) (Figure. 4).

Table 4. ^{1}H NMR, ^{13}C NMR, HSQC and HMBC data of compound **2**

Position	**δ H**	**δ C**	**HSQC**	**HMBC**
1	—	122.0		—
2	7.58 *d (J = 5)*	112.5	C-H	C 1, C3, C 4, C 6 and C 7.
3	—	147.1	—	—
4	—	151.2	—	—
5	6.93 *d (J =5)*	114.6	C-H	C 1, C3, C 4 and C 6
6	7.62 *dd(J=10)*	124.0	C-H	C 1, C2, C 4 C 5 and C 7
7	—	166.5	—	—
CH_3O	3.94	55.4	C-H	C 3 and C 4
3-OH	—	—	—	—
7-OH	—	—	—	—



A B C

Figure 4HSQC (A) and HMBC (B) correlation of H to C of compound **2**

Characterization of compound 3

Compound **3**was isolated as yellow amorphous solid (30% ethyl acetate in n-hexane).The IR spectrum (Appendix **3A**) showed absorption bands at 3391,1659 and 1476 cm^{-1}corresponding to the stretching vibration peak of the hydroxyl (OH),conjugated carbonyl (C=O) groups, and aromatic moieties, respectively.The UV vis spectrum (Appendix**3B**) (in MeOH) of compound **3**showed absorption bands of λ_{max}at 244, 263, 319, 374 nm.

^{1}H NMR spectrum (Appendix **3C**) displayed resonances for two aromatic methoxyls (δ 3.91 and 3.92), a hydroxyl (δ 13.05), a three-proton spin system (δ7.45, 7.24 and 7.4; *J*=8 and 4 Hz) and an AB system (δ7.59 and 7.36) of two ortho-coupled protons (*J*=8 Hz). The ^{13}C NMR spectrum(Appendix **3D**) indicated that the aromatic methoxyl at δ 61.28 and 55.24(Table **5**)and that there were no substituted groups at either a C-1 or C-8 position according to the carbonyl carbon shift δ175.00[56].

The DEPT-135 spectrum (Appendix **3E**) showed seven carbon signals only positively, comprising five methine C in the aromatic chemical shift and two methyl C in the methoxy chemical shift. Therefore, based on the spectroscopic data and comparison of this data with the literature [57], the compound was identified to be 3, 4-dimethoxy-7-hydroxyxanthone (**3**) (Figure **5**).

Table 5. ^{1}H NMR and ^{13}C NMR data of compound **3**and comparison with the Reported [57].

Position	δ ^{1}H Observed*J*	δ ^{1}H Reported	δ ^{13}C Observed	δ ^{13}C Reported
1	7.59*d*(8)	7.6 *d* (9.2)	123.1	122.0
2	7.36*d* (8)	7.35 *d* (9.2	113.7	115.8
3	—	—	150.5	150.5
4	—	—	146.4	148.4
4a	—	—	145.0	147.4
4b	—	—	149.9	148.5
5	7.45*d*, (8)	7.44*d* (9)	119.0	118.8
6	7.24*dd*, (8, 4)	7.25 *dd* (9.0, 3.0)	123.9	124.0
7	—	—	155.9	153.6
8	7.4*d* (4)	7.42*d* (3)	105.8	108.5
8a	—	—	122.3	120.6
8b	—	—	115.8	113.2
C=O	—	—	175.00	175.1
MeO-3	3.74	3.86	55.2	56.7
MeO-4	3.72	3.80	61.3	60.9
HO-7	13.05	9.8	—	—

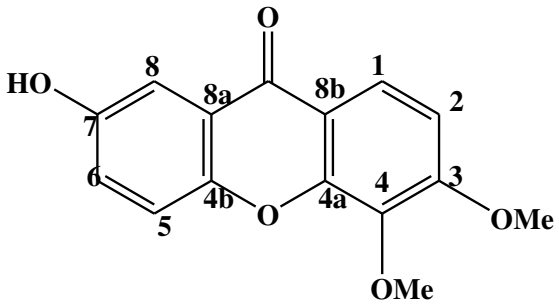


Figure 5. The structure of compound **3**

4.5Evaluation of the results of antibacterial activity.

The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with the ruler and the results of antibacterial activity was recorded (Table **6**) was shown below.

Table 6. Zone of inhibition of the extracts (200mg/mL) and compound **3** (50mg/mL) in mm

Test organism	Hex extract	Chl extract	Ac extract	Meth extract	Cpd**3**	Gent	DMSO
E. coli	12	30	30	34	15	26	NI
p. aeruginosa	10	11	32	28	11	20	NI
S. aureus	NI	12	26	26	10	20	NI
B. subtilis	15	20	32	30	10	15	NI

*Key: Hex= hexane, Chl = chloroform, Ac = Acetone, Meth = Methanol, Cpd**3** = Compound **3,** Gent* **=** *Gentamicinand NI = no inhibition*

As shown in Table 6, acetone extract showed the greatest activity in both Gram positive and Gram negative bacteria. It was highly potent against *p.aeruginosa,B. subtilus, E. coli,and S.*

*aureus*by showing inhibition zone of32, 32, 30 and 28mmrespectively. Methanol extract showed greaterinhibition zoneagainst *E. coli,B. subtilus,P. aeruginosa* and*S. aureus*by showing inhibition zone of 34, 30,28 and 26 mm respectively.

On the other hand, the chloroform extract was showed moderate activity against *E. coli, B. subtilus, P. aeruginosa* and*S.aureus*by showing inhibition zone of 30, 20,11 and 12 mm respectively. But relatively the hexane extract and compound **3** showed activity less than the average value of the reference drag against *E. coli, B. subtilus, P. aeruginosa* and*S.Aureus*by showing inhibition zone of 12, 15 and 10 mm for hexane and 15, 10, 11 and 10 mm for compound **3**respectively.

The better activity of both acetone and methanol was greater than the observed activity of the reference drug (Gentamycin) as demonstrated by the observed inhibition zone values (Table **6**and **Appendix 4A**). The better antimicrobial activity of acetone and methanol extract could account the synergetic interaction of secondary metabolites in the extract compared to positive standard control; additionally, the better activity of acetone and methanol extracts were due to their high constituent of bioactive secondary metabolites like alkaloids, flavonoids, phenols, Saponins, Terpenoids and Quinones in their extract as shown in Table **2**.

Chloroformextract shows better activity against *E. coil* and moderate activity against *Bacillussubtilus*with inhibition zone of 30 and 20 mm respectively. However, it has low activity against both *P.aeruginosa* and *S. aureus* by inhibiting 11 and 12 mm diameter.

Hexane extract showed the lowest activity against all the tested strain, compared to positive control (gentamycin), other extracts and compound **3.** The low activity of hexane extract could be related to the absence of polar bio active secondary metabolites, since hexane interact only with nonpolar organic molecules.

The high antimicrobial activity of Securidaca longepedunculata roots extracts were also reported by [39, 46] which attributed these activities to the presence of flavonoids and Saponins.

Flavonoids are known to be synthesized by the plants in response to microbial infection in nature, so it is not surprising in this research that they are very effective against a wide array of microorganisms [58].This activity is probably due to their ability to complex with extracellular and soluble proteins and other components of cell walls. Similarly, the lipophilic nature of flavonoids may also disrupt microbial membrane [59].

Compound **1**and **2** werenot obtained in a sufficient amount to allow bacterial testing. Compound**3**(3, 4-dimethoxy-7-hydroxyxanthone) showed a moderate activity against both gram negative and positive bacteria as shown in Table **6.**

5. Conclusion and Recommendation.

5.1 Conclusion

The present investigation revealed that the extract of *Securidaca longipedunculata* have potent antimicrobial activity which explains its use in traditional system of medicines.These four extract and isolated compound were analyzed for their antimicrobial activity against four bacterial strains and phytochemical was carried out to determine the secondary metabolites in each extract. The antimicrobial analysis showed that the acetone extract was the most active with the mean zone of inhibition 30mm followed by methanol extract 28 mm, chloroform 16 mm and hexane extract 8mm. Compound**3**showed moderate antibacterial activity with 12 mm mean zone of inhibition.

Phytochemical screening revealed that terpenoids, Coumarins and Saponins are present in the entire extract, Alkaloids and Quinone were present in acetone and methanol extract only, and Phenols and Flavonoids were present in all the extract except hexane extract. The presence of these secondary metabolites accounts for the antimicrobial activities of the extracts and also, justified the claimed traditional medicine use in the treatment of bacterial infections.

The present study has identified the isolation and characterization of three compounds, two of them were new compounds, compound 1 (3-hydroxy benzoic acid) and compound **3** (3, 4- di methoxy-7- hydroxyxanthone) for the first time from the *S. longipedunculata* species. The antimicrobial activity of compound **1**and compound **2** were not investigated, because they were

not obtained in sufficient amount to conduct their antibacterial activity.Compound**3** displayed potent and relevant pharmacological activities with considerable antibacterial activity against selected Gram positive and Gram negative bacteria.The results obtained show that the compound could be of pharmaceutical interest for therapeutically application as complementary antibacterial agents for infectious disease. Therefore antimicrobial activity of all the plant extracts and isolated compound against the different clinical strains of bacteria supported the scientific validity of the plant being used traditionally as a medicine.

5.2 Recommendation.

Further work is recommended on crude extracts and bioactive compounds to investigate its structures and indicate their exact potential to inhibit several pathogenic microbes and development of a novel broad spectrum antimicrobial herbal formulation.

The present study use only acetone extract for the isolation of pure compounds, therefore,further isolation and characterization of bioactive compounds from the methanol, chloroform and hexane extracts is recommended.

More biological assay on other strain need to be conducted on extracts and isolated compounds so as to stablish the traditional use of plants.

It is recommended that toxicity test should be carried out on the crude extract and isolated compounds.

Reference

1. Farnsworth NR. Ciba foundation symposium. Ethno pharmacol and drug development. *J.Ethno-pharmacol.***1994,***185***,** *45-59*
2. UNESCO, World Decade for Cultural Development; World Health Organization: Culture and Health: orientation texts on the France 1996 theme. pp.129
3. Promotion of Ethno botany and the Sustainable Use of Plant Resources in Africa, Terminal Report. UNESCO, 1998, p 60.
4. Lambert, J. Ethiopia: Traditional medicine and the bridge to better health. World Bank, available at: http://www.world Bank. rog/afr/ik/dfefault.htm.
5. Kebede, D.; Alemayehu, A.; Binyam, G.; Yunis, M. A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiop. J.Health Dev*. **2006**, *2,*128-134.
6. Goh, S.; Chuah, C.; Mok, J. *Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases*; Pelanduk, 1995, pp 83.
7. Getahun, T.; Reneela, P.; Aman, D. Isolation and characterization of natural products from *Helinusmystachnus*. *J. Chem. Pharm. Res*. **2012**, *4,* 1756-1762.
8. Kirtikar, K. R. *Indian Medicinal Plants*; International Book, India: Dehradun, 1995, pp830- 832.

9. Baris, O.; Gulluce, M.; Sahin, F.; Ozer, H.; Kilic, H. et al. Biological activities of the essential oil and methanol extract of AchilleabiebersteiniiAfan. (Asteraceae). *Turk J.Biol* .**2006**, *30, 65-73*.
10. Hammer, K.; Carson, C.; Riley, T. Antimicrobial activity of essential oils and other plant extracts. *J. App. Microbiol.***1999**, *86, 985-990*.
11. Gibbons, S. An overview of plant extracts as potential therapeutics. Expert Opinion therapeutic patents **2003,***13, 489-497*
12. Addis, G.; Abebe, D.; Urga, K. A survey of traditional medicine in Shirka District, Arsi Zone, Ethiopia. Etheno-pharm.**2001,** *19, 30-47*.
13. Asfaw, D.; Abebe, D.; Urga, K. Traditional medicine in Ethiopia: perspectives and developmental efforts. *J.Ethiop Med*. **1999,***1,* 114-17.
14. Boominathan, M.; Ramamurthy, V. Antimicrobial activity of *Heliotropiumindicum* and *Coldeniaprocumbens*. *J. Eco boil.* **2009***, 24,*11-15.
15. Wallnofer, B. A new species of *Securidaca* L. (Polygalaceae) from Peru. AnnalendesNaturhistorischen Museums in Wien. **1998**, *100,* 709-714.
16. Yang, X.D.; Xu, L.Z.; Yang, S.L. Xanthones from the stems of *Securidaca inappendiculata*. *Phytochemistry*. **2001a,***58*, 1245-1249
17. Da Costa, C.S.; De Aguiar-Dias, A.C.A.; Simoes, A.O. *Securidaca marajoara*(Polygalaceae), a new species from the Brazilian Amazon. *Phytotoxic*. **2013,** *137,* 53-56.
18. Van Wyk, B.E.; Van Oudtshoorn, B.; Gericke, N. *Medicinal plants of South Africa*, second ed. Briza: Pretoria, 2009, pp 268.
19. Van Wyk, B.E.; Van Oudtshoorn, B.; Gericke, N. *Medicinal plants of South Africa*, first ed. Briza: Pretoria, 2005, pp 236.
20. Coates-Palgrave, M. Keith Coates Palgrave Trees of Southern Africa, Third Edition, Struik Publishers: Cape Town,2005, pp 458-459.
21. Baloyi, O.; Tshisikhawe, M.P. The population ecology of *Securidaca longepedunculata* Fresen. *South Afri J. of Bota*. **2009**, *75*, 430.

22. Baloyi, O.; Bhat, R.B.; Tshisikhawe, M. P. The germination and propagation potential of *Securidaca longepedunculata* Fresen. *South Afri J. Bota*. **2012**, 79, 177.
23. .Maroyi, A. Traditional use of medicinal plants in South-central Zimbabwe: Review and perspectives. *J. of Ethno Ethno medic*. **2013**, *9*, 31 http://www.ethno biomed.com/content/9/1/31.
24. Nadembega, P.; Boussim, J.I.; Nikiema, J.B.; Poli, F.; Antognoni, F. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: *An ethnobotanical study. J. of Ethno pharmacol*. **2011,***133*, 378-395.
25. Semenya, S.S.;Potgieter, M.J. Ethnobotanical survey of medicinal plants used by Bapedi traditional healers to treat erectile dysfunction in the Limpopo Province, *South Africa. J. Med. Plants Res*. **2013**, *7*, 349-357.
26. Motlhanka, D. M. T.; Nthoiwa, G. P. Ethnobotanical survey of medicinal plants of Tswapong North, in Eastern Botswana: A case of plants from Mosweu and Seolwane villages. *European J. Med. Plants.***2013***3*, 10-24.
27. Mustapha, A. A. Ethno-medico-botanical uses of *Securidaca longepedunculata* (Fresen) (Family Polygalaceae) from Keffi local government,Nasarawa State, Nigeria. *J. Nat Remedies*. **2013a,***13*, 133-137.
28. Mustapha, A.A. Ethno-medicinal field study of anti-fertility medicinal plants used by the local people in Keffi local government, Nasarawa State, Nigeria. *Int. J. of Med Plant Res*. **2013b**. *2*, 215-218.
29. Borokini, T.I.; Clement, M.; Dickson, N.J.; Edagbo, D.E. Ethnobiological survey of traditional medicine practice for circulatory and nervous system related diseases in Oyo State, Nigeria. *Top class J. Herbal Med*. **2013,***2, 111-120*
30. Nadembega, P.; Boussim, J.I.; Nikiema, J.B. Poli, F.; Antognoni, F. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. *J. of Ethno pharmacol*. **2011**, *133*, 378-395.

31. Muanda, F.N.; Dicko, A.; Soulimani, R. Assessment of polyphenolic compounds, in vitro antioxidant and anti-inflammation properties of Securidaca longepedunculata root barks. C. R. biology.**2010,** *333*, 663-669.
32. Junaid, S.A.; Abubakar, A.; Ofodile, A.C.; Echeonwu, G.O.N.; Okwori, A.E.J.;Ajetunji, J.A. Evaluation of *Securidaca longipedunculata* leaf and root extracts for antimicrobial activities. *Afr. J. Microbiol Res*. **2008,***2*, 322-325.
33. Haruna, Y.; Elinge, C.M.;Peni, I.J.; Dauda, D.; Aiki, F.*In vivo* trypanocidal effect of aqueous root extracts of *Securidaca longepedunculata* and its phytochemical analysis. *Afr. J. Phar. and Pharmacol*. **2013a**, *7,* 2838- 2842
34. Auwal, S.M.; Atiku, M.K.; Wudil, A.M.; Sule, M. S. Phytochemical composition and acute toxicity evaluation of aqueous root bark extract of Securidaca longepedunculata (Linn). Bajopas. **2012**, 5, 67-72.
35. Adebayo, O. L.; Osman, K. A comparative evaluation of in vitro growth inhibitory activities of different solvent extracts of some medicinal plants in Northern Ghana against selected human pathogens. *J. Pharmacy.* **2012***, 2, 199-206*.
36. Meyer, J.M.M.; Rakuambo, N.C.; Hussein, A.A: Novel xanthones from *Securidaca longepedunculata* with activity against erectile dysfunction. J. of Ethno-pharmacol. **2008** 119, 599-603.
37. Lannang, A.M.; Lontsi, D.; Ngounou, F.N.; Sondengam, B.L.; Nkengfack, A.E.; van Heerden, F.R.; Assob, J.C.N: Securidaca xanthone A, a hepato oxygenated xanthone from *Securidaca longepedunculataFitoterapi*a. **2006**, *77*, 199-202.
38. De Tommasi, N.; Piacente, S.; de Simone, F.; Pizza, C. New sucrose derivatives from the bark of *Securidaca longipedunculata*. *J. Nat. Prod*.**1993**,*56***,**134-137.
39. Arnold, H. J.; Gulumian, M. Pharmacopoeia of traditional medicine in Venda. J. Ethnopharmacol.**1984,***12,* 35-74.

40. Chhabra, S. C.; Mahunnah, R. L. A; Mshiu, E. N. Plants used in traditional medicine in eastern Tanzania. V. Angiosperms (Passifloraceae to Sapindaceae). *J. Ethnopharmacol.* **1991**, *33*,143- 157.
41. Desta, B. Ethiopian traditional herbal drugs. Part II: Antimicrobial activity of 63 medicinal plants. *J. Ethnopharmacol*.**1993,***39,* 129-139
42. Ndamitso, M.M.; Mohammed, A.; Jimoh, T.O.; Idris, S.; Oyeleke, S.B.; Etsuyangkpa, M.B. Phytochemical and antibacterial activity of *Securidaca longepedunculata* on selected pathogens. Afr.*J*.Microbiol Res. **2013**, *7,* 5652-5656
43. Musa, A. A.;Oyewale, A.O.; Ndukwe, I.G.; Yakubu, S. E.; Abdullahi, M.S. Phytochemical screening and antimicrobial activity of solvent fractions of *Securidacalongepedunculata* (Fresen) root bark methanol extract. *J.Chem Pharma Res*. **2013**, 5, 28-33.
44. Adebayo, O.L.; Osman, K. A comparative evaluation of in vitro growth inhibitory activities of different solvent extracts of some medicinal plants in Northern Ghana against selected human pathogens. *IOSR J. Phar*.**2012**,*2*, 199-206.
45. Ndamitso, M.M.; Mohammed, A.; Jimoh, T.O.; Idris, S.; Oyeleke, S.B.; Etsuyangkpa, M.B. Phytochemical and antibacterial activity of *Securidaca longepedunculata* on selected pathogens. *Afr. J. Microbiol Res*. **2013**, *7*, 5652-5656
46. Ajali, U.; Chukwurah, B.K. C. Antimicrobial activity of *Securidaca longipedunculata*. Phytomed.**2004**,*11*, 701-703.
47. Alitonou, G.A.; Koudoro, A.Y.; Dangou, J. S.; Yehouenou, B.; Avlessi, F.; Adeoti, S.; Menut, C.; Sohounhloue, C.K. Volatile constituents and biological activities of essential oil from *Securidaca longepedunculata* Fresen. Growing in Benin. Scientific Study and Res.**2012**,*13*, 033-042.
48. Karou, S.D.; Tchacondo, T.; Tchibozo, M.A.D.; Anani, K.; Ouattara, L.; Simpore, J.; de Sousa, C. Screening of Togolese medicinal plants for few pharmacological properties. Pharmacognosy Res.**2012,***4*, 116-122.

49. Bah, S.; Jäger, A.K.; Andersen, A.; Diallo, D.; Paulsen, B.S. Antiplasmodial and GABA A-benzodiazepine receptor binding activities of five plants used in traditional medicine in Mali, West *Afr. J. Ethno pharmacol*.**2007**, *110*, 451- 457.

50. Haruna, Y.; Kwanashie, H.O.; Anuka, J.A.; Atawodi, S.E.; Hussaini, I.M.*In vivo* anti-malarial activity of methanol root extract of *Securidaca longepedunculata* inmice infected with *Plasmodium bergei*. *It. al. J. Modern Biol*. **2013c**,*3*, 7-16.
51. Ancolio, C.; Azas, N.; Mahiou, V.; Ollivier, V.; Di Giorgio, C.; Keita, A.;Timon-David, P.;Balansard, G. Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. *Phytotherapy Res*.**2002**,*16*, 646 649.
52. Kassa, M.; Mshana, R.; Regassa, A.; Assefa, G. *In vitro* test of five Ethiopian medicinal plants for antimalarial activity against Plasmodium falciparum. *Ethiopian J. Science*.**1998**,*21*, 81-89.
53. Sofowora A. Medicinal plants and Traditional medicine inAfrica. Spectrum Books Ltd, Ibadan, Nigeria; **1993**; pp191-289.

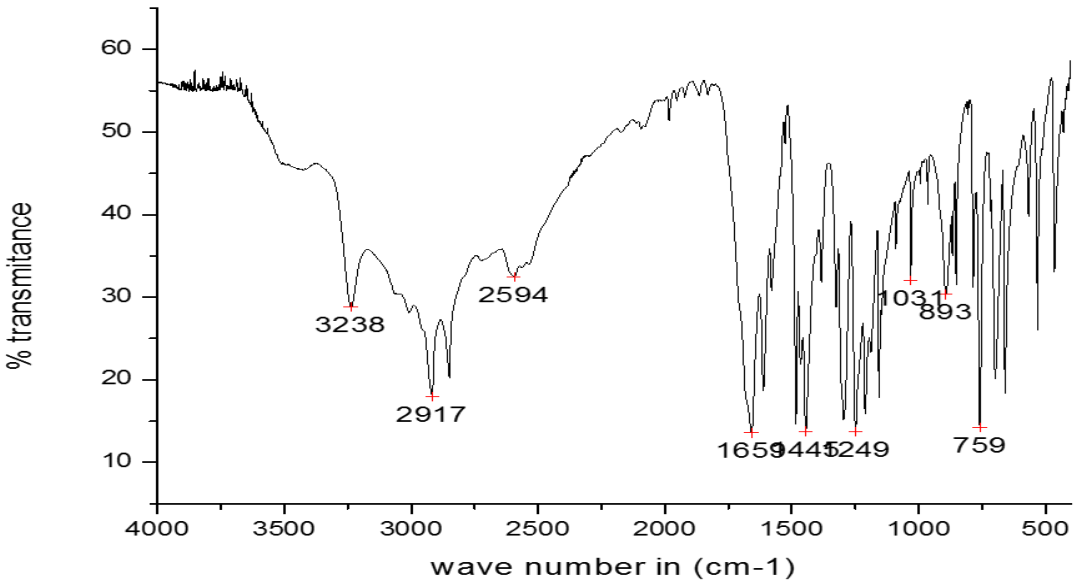
54. Harbourne,J. B.Phytochemical Methods*: Guide to Modern Techniques of Plant Analysis*. 3rd edition, Chapman and Hall, London, 1998,pp83-90.

55. Singh, B.;Sahu, P. M.; Sharma, M. K. Anti-inflammatory and Antimicrobial activities of triterpenoids from *Strobilanthescallosus*Nees. Phytomed.**2002**, **9:** 355-359.
56. Philip, W. W.;Sarath, P G.; Uvais, M.; Sultanawa, S.; Rymantas, K. Carbon -13 NMR study of naturally occurring xanthones. *Org MagnResonance*. **1977**, *9,*631-636
57. Yang, X.D.; Xu, L.Z.; Yang, S.L. two new Xanthones from the stems of *Securidaca inappendiculata*. *Acta Botanica Sinca*. **2003,***45*, 365-368.
58. Bennett, R N.;Walls grove, RM. Secondary metabolites in plant defense mechanisms. *New Phytol*. **1994**; 127: 617-633.

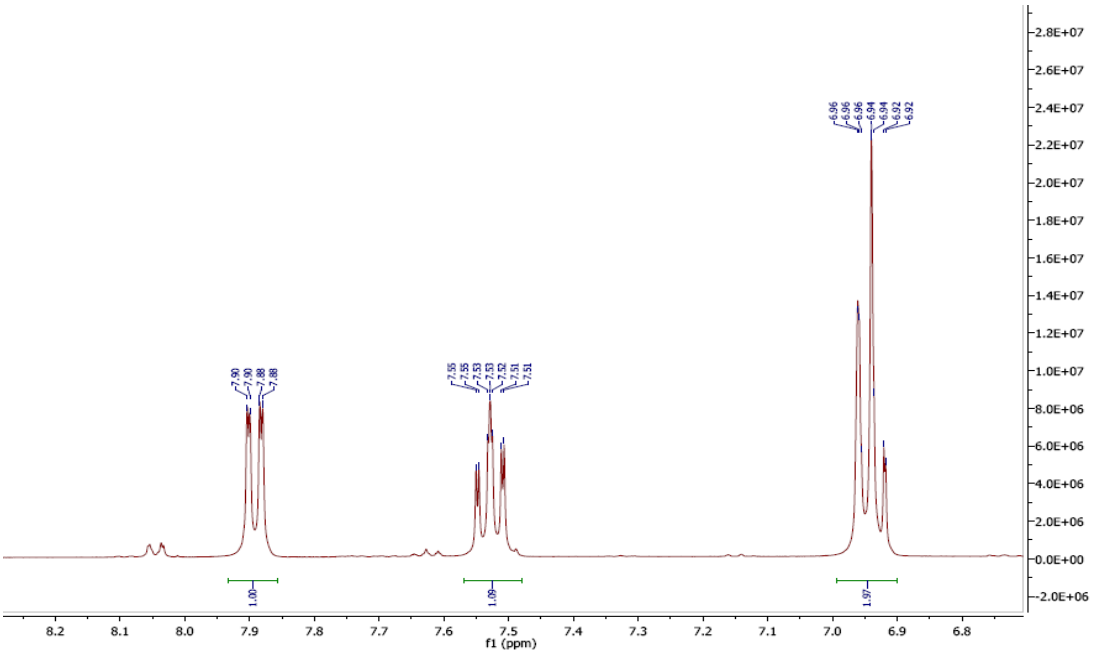
59. Tsuchiya, H.; Sato, M.; Miyazaki T.; Fujiwara, S.;Tanigaki, S.;Ohyama, M.;Tanaka, T.;Iinuma, M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol*. **1996**, *50*, 27-34

Appendixes

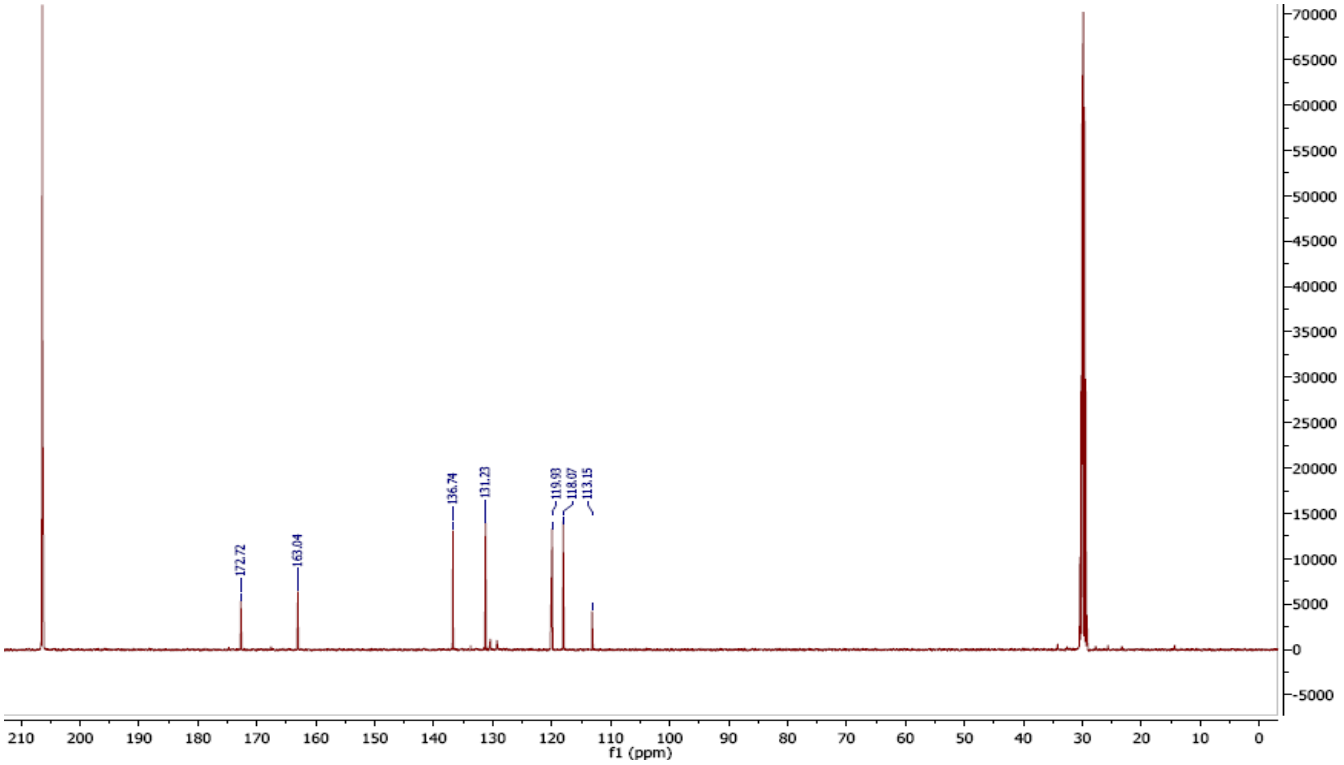
Appendix 1



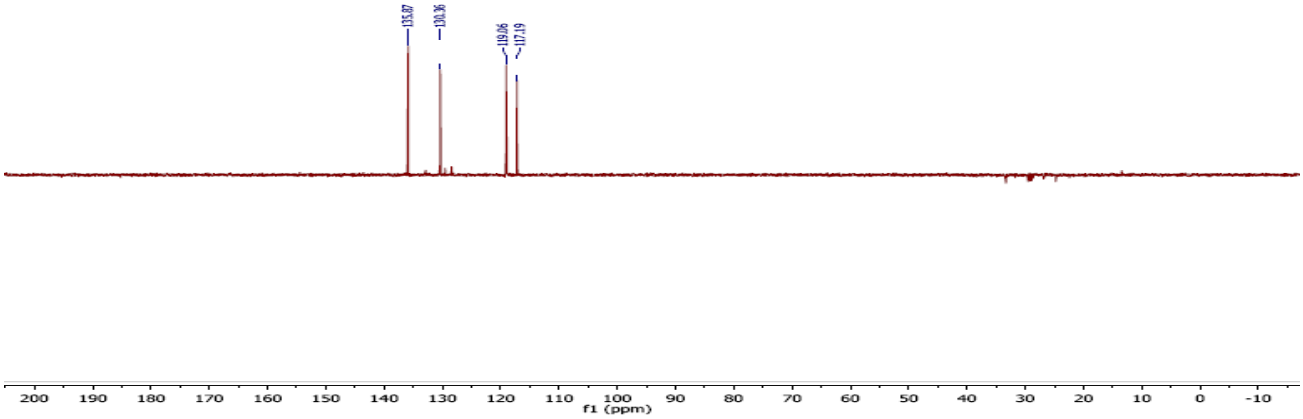
The IR spectra of compound **1**



The ^{1}H NMR(acetone, 400M Hz) spectra of compound **1**

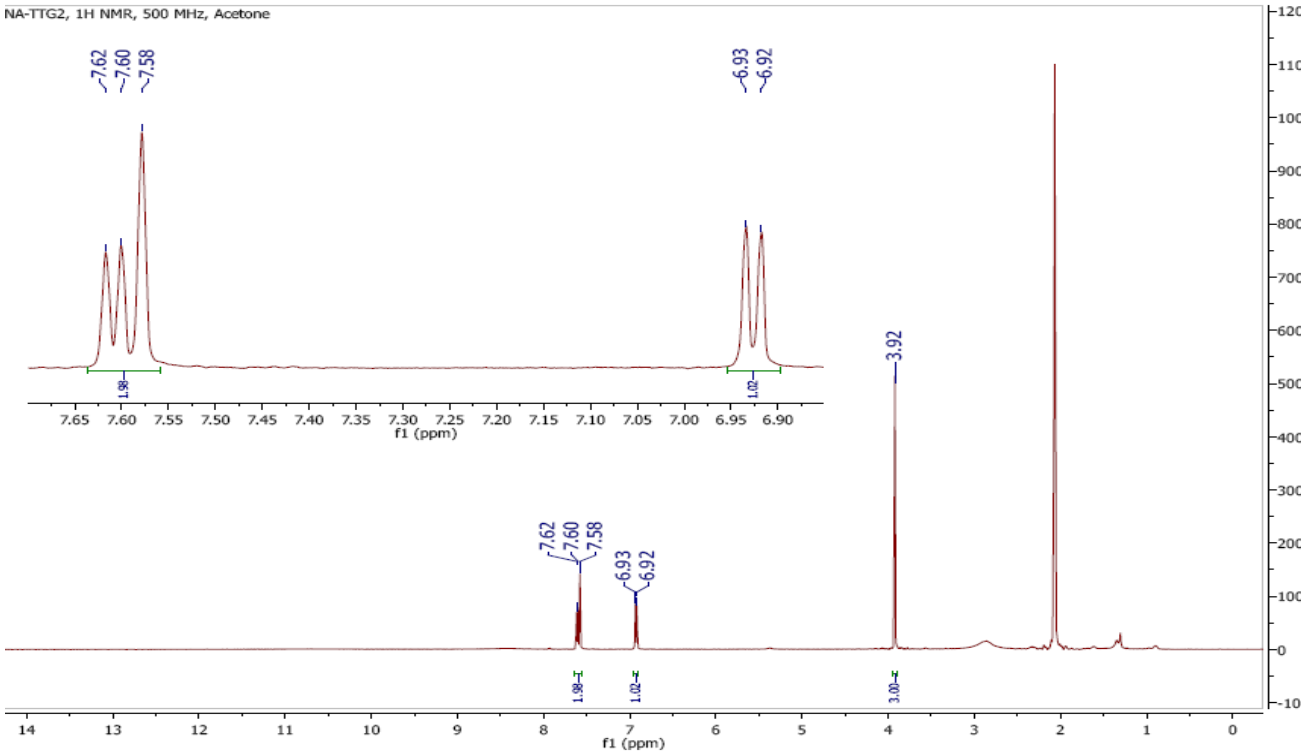


The ^{13}C (acetone, 100M Hz) spectra of compound **1**

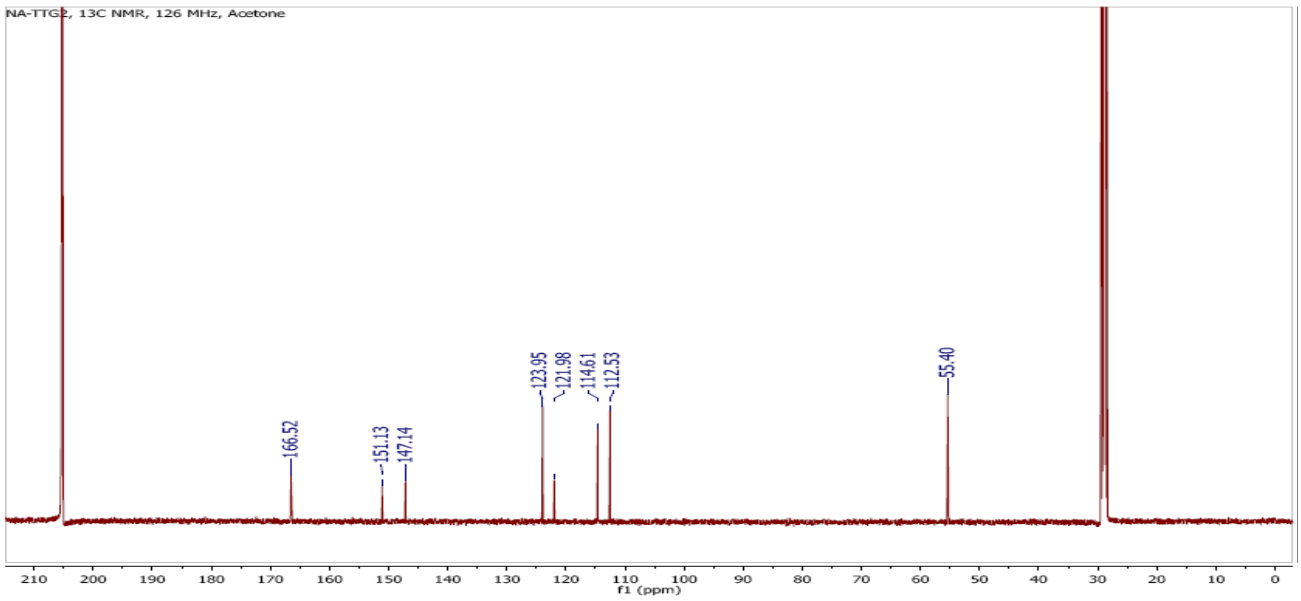


The DEPT-135 (acetone, 100M Hz)spectra of compound **1**

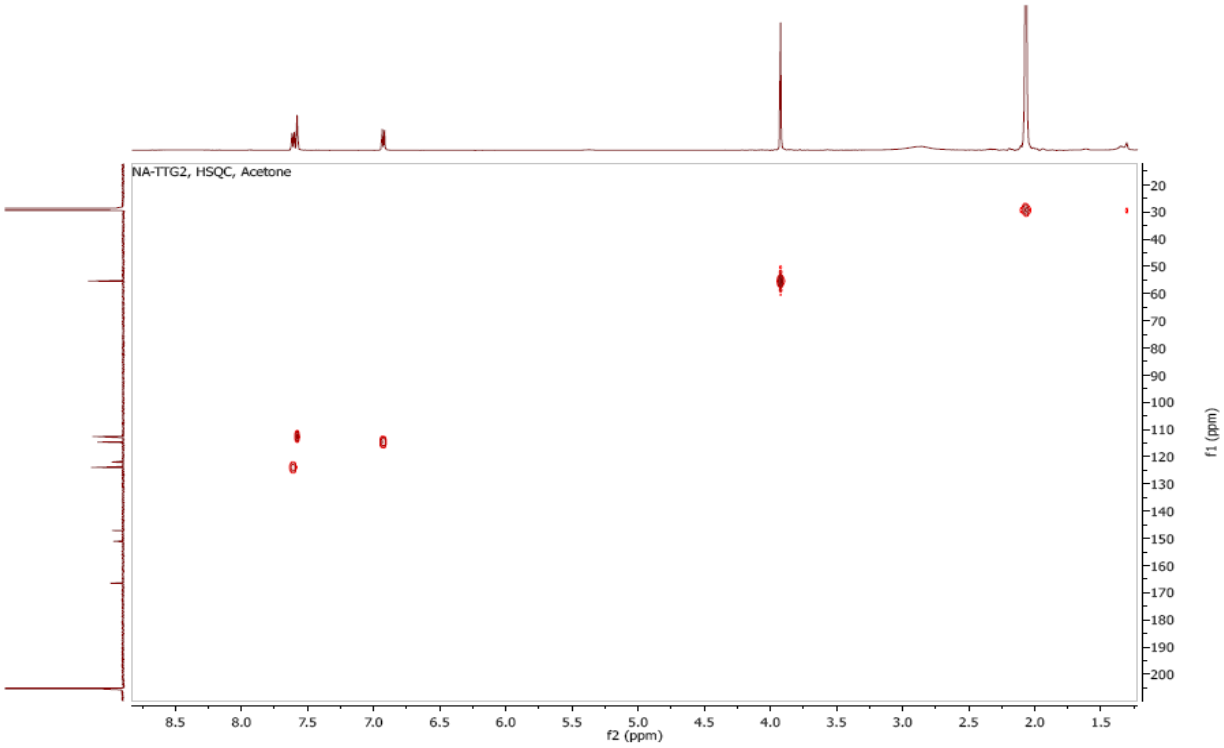
Appendix 2



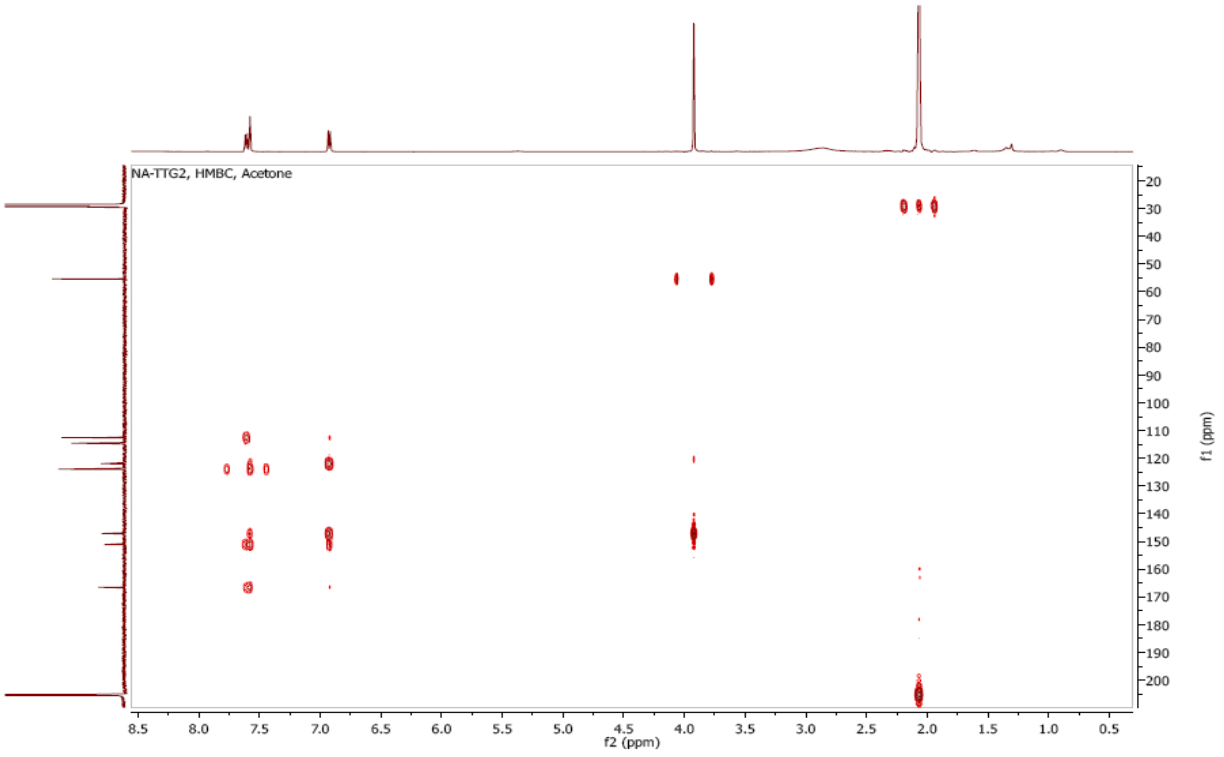
[1] H NMR spectra of compound 2



[13] C NMR spectra of compound 2

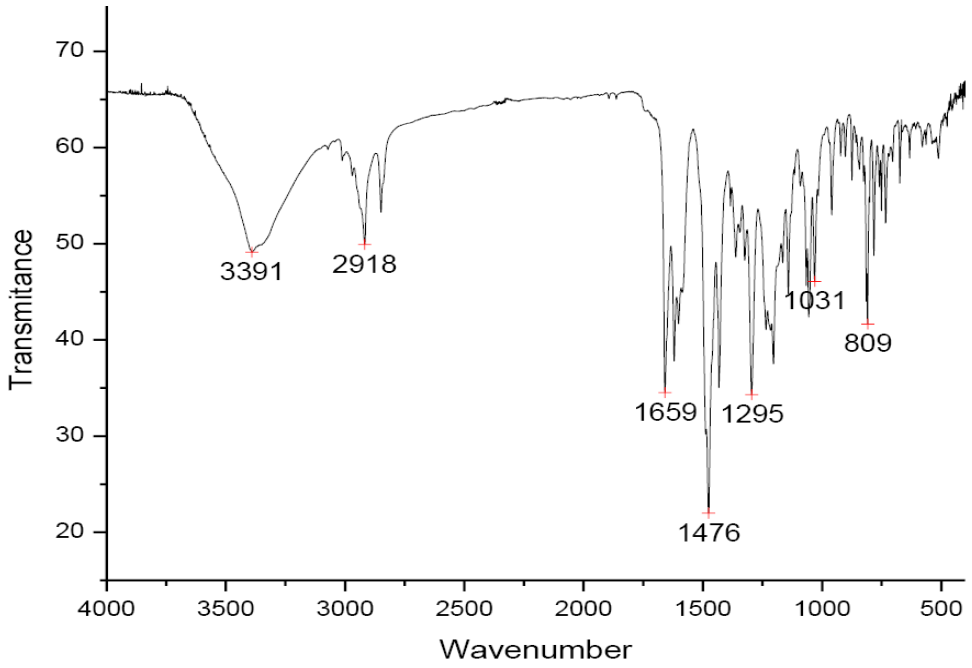


HSQC spectra of compound -2

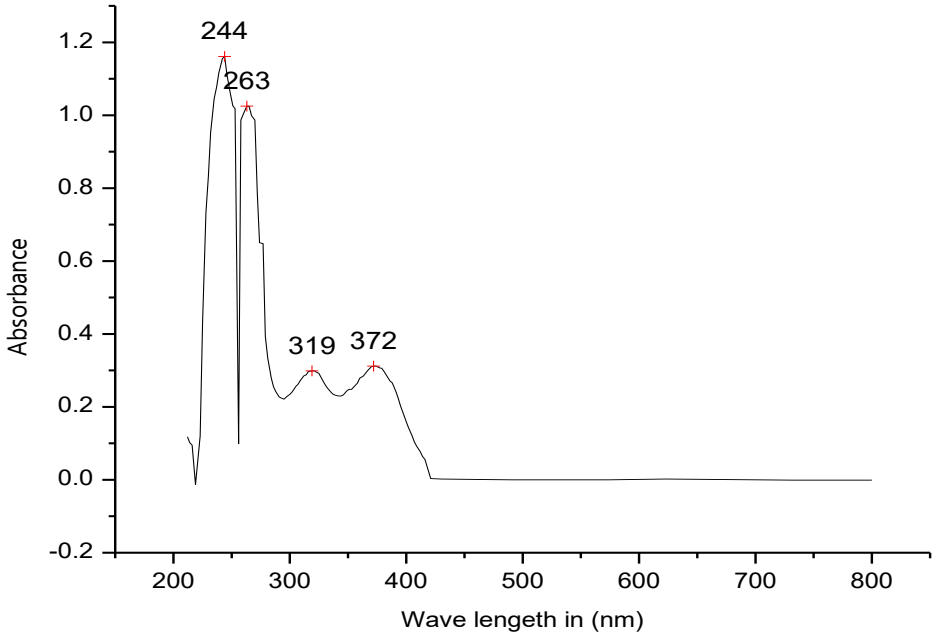


HMBC spectra of compound -2

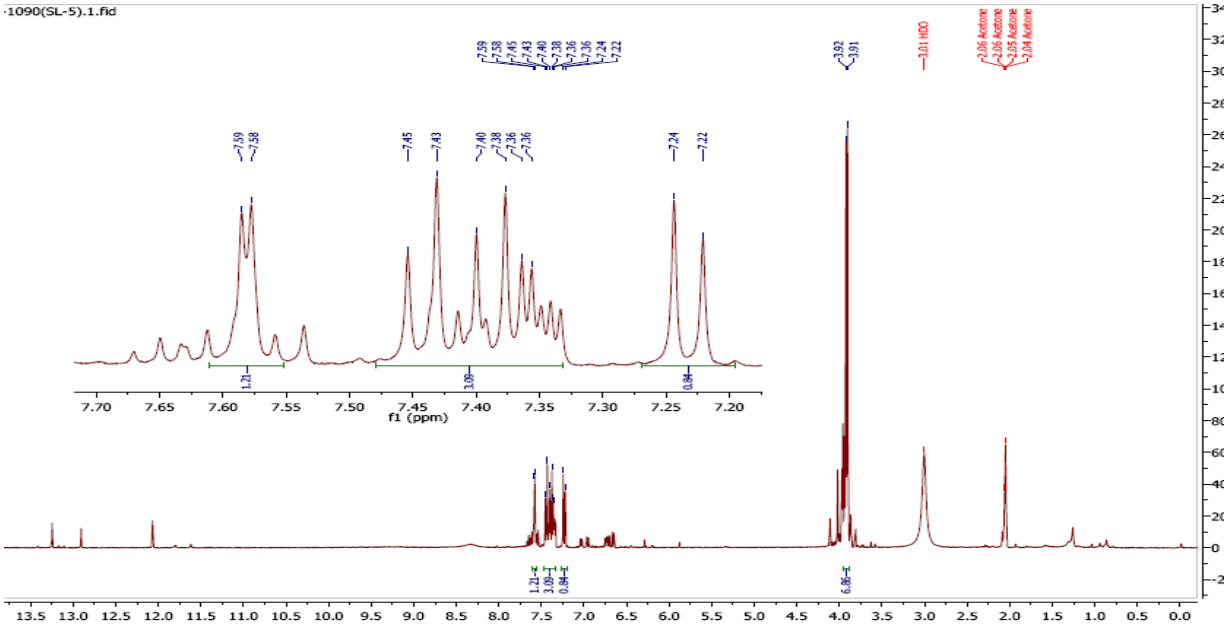
Appendix 3



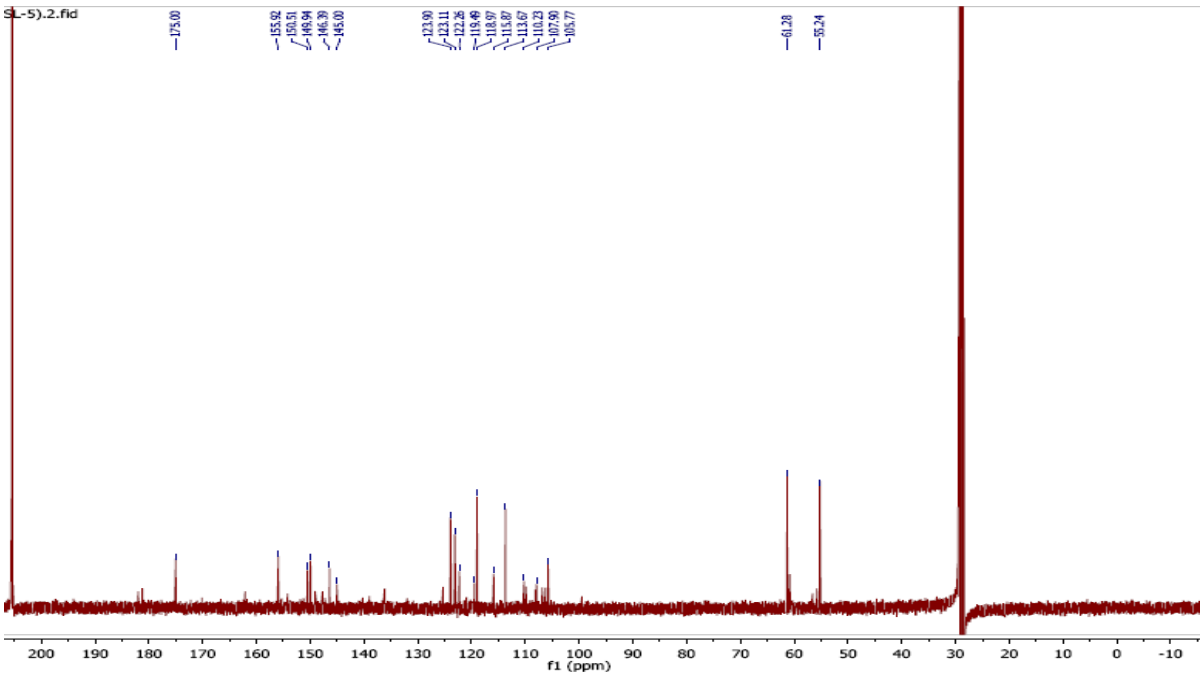
The IR spectra of compound **3**



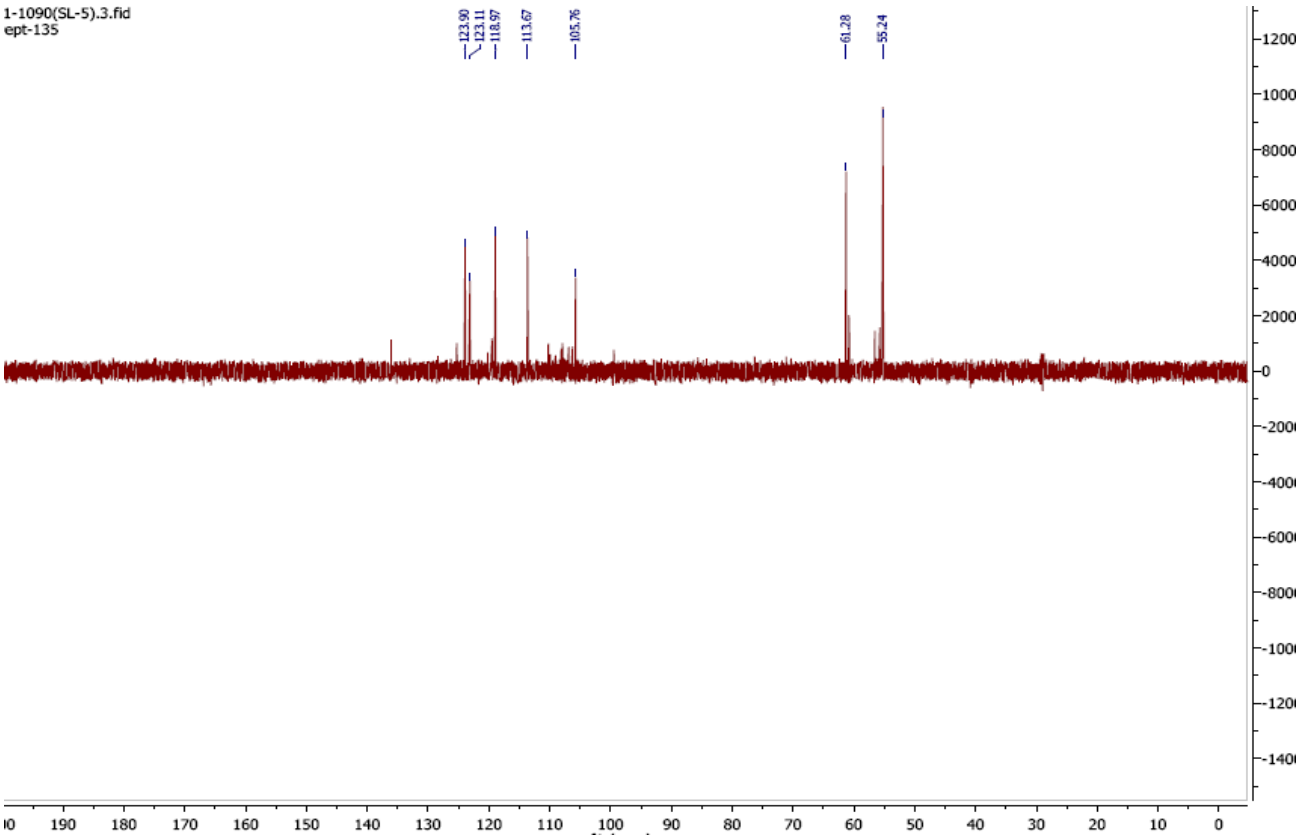
UV spectra of compound 3



The[1]H NMR (acetone, 400M Hz)spectra of compound **3**

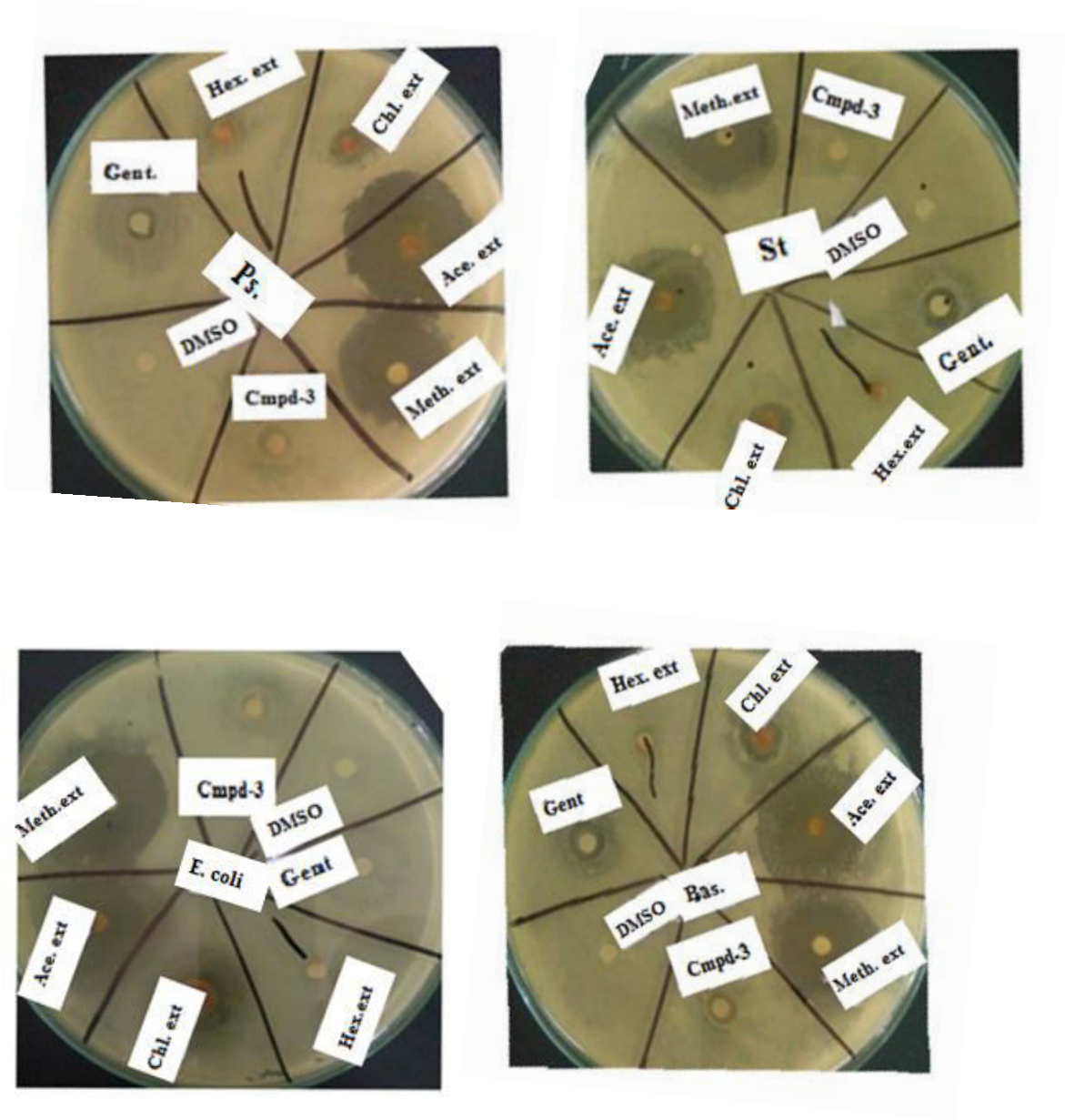


The [13]C NMR(acetone, 100M Hz) spectra of compound **3**



The DEPT-135(acetone, 100M Hz) spectra of compound **3**

Appendix 4



Bio activity result of extracts and isolated compound.