PHYTOCHEMICAL ANALYSIS OF THE ROOTS OF *SYZYGIUM GUINEENSE* FOR ANTIBACTERIAL ACTIVITY

BY

Ayele Bekele

A MASTER THESIS SUBMITTED TO THE OFFICE OF GRADUATE STUDIES OF ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY, SCHOOL OF APPLIED NATURAL SCIENCE DEPARTMENT OF APPLIED CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED CHEMISTRY

Adama, Ethiopia

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ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCE

APPLIED CHEMISTRY DEPARTMENT

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Advisor: Milkyas Endale (PhD)

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Adama, Ethiopia

September 17, 2018

APPROVAL OF BOARD OF EXMINERS

We, the undersigned, member of the board of examiners of the final open defense by Ayele Bekele have read and evaluated his thesis entitled **" PHYTOCHEMICAL ANALYSIS OF THE ROOTS OF SYZYGIUM GUINEENSE FOR ANTIBACTERIAL ACTIVITY"** and examined the candidate. This is therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

Advisor	Signature	Date
Co-Advisor	Signature	Date
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Chairperson	Signature	Date
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Internal Examiner	Signature	Date
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DECLARATION

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other institution, and all sources of material used for this thesis have been duly acknowledge.

Name: Ayele Bekele Signature: _____

This MSc thesis has been submitted for examination with our approval as thesis advisors.

Dr. Milkyas Endale (PhD) _____

Advisor Signature

Dr. Yadessa Melaku _____

Co-Advisor Signature

Date of submission _____

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ACRONYMS AND ABBREVIATIONS

TMS	Tetramethylsilane
TLC	Thin layer chromatography
UV	Ultraviolet
TSB	Tripticase Soy Broth
DMSO	Dimethyl sulfoxide
DRC	Democratic Republic of Congo
Glc	Glucose
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
DEPT	Distortion less Enhancement by Polarization Transfer
^{1}H-NMR	Proton-Nuclear Magnetic Resonance
$CDCl_3$	Deuterated Chloroform
IR	Infrared
DCM	Dichloromethane
MeOH	Methanol
EtOAc	Ethyl acetate
ATCC	American Type Culture Collection
WHO	World Health Organization
R_f	Retardation Factor
CC	Column Chromatography
MHA	Muller Hinton Agar
d	Doublet
s	Singlet
t	Triplet
m	Multiplicity
ppm	Parts per million
J	Coupling constant
Hz	Hertz

ABSTRACT

Syzygium guineense (Myrtaceae) is widely used in folk medicine in different countries for the treatment of various ailments including stomachache, dysentery, diarrhea, hypertension, leprosy, gonorrhea, hemorrhoid, snack bit, malaria, tuberculosis and liver problem. In view of its traditional uses, however, to the best of our knowledge, there was no phytochemical study that has been carried out to identify the active metabolites from the roots of Syzygium guineense. In this report, an attempt was made to explore the chemicals and to evaluate antibacterial activities of the solvent extracts and isolated compounds of the roots of S. guineense.

*In this regard the powdered roots (500 g) of S. guineense were successively extracted with dichloromethane/methanol (1:1) (4L) and methanol (2.5L) at room temperature on maceration to give 1.90% and, 5.24% respectively. Phytochemical screening tests revealed the presence of terpenoids, saponins, flavonoids, tannins, alkaloids, phenols and glycosides in both roots extracts. Silica gel column chromatographic separation of the roots extracts of dichloromethane/methanol (1:1) afforded arjunolic acid (**5**) whereas the methanol extract afforded compound (**18**) which is derivative of steroid lupeol (**17**). Structural elucidations of these compounds were done by employing IR and NMR and comparison with literature data. The extracts and isolated compounds were also evaluated for their antibacterial activities by disc diffusion method against four bacterial strains; one gram positive staphylococcus aureus and three gram- negative Escherichia coli, Klebsiella pneumoniae, and Salmonella typhi.*

DCM: MeOH and MeOH extracts both showed significant antibacterial activities against the four bacterial strains at concentrations of 200 μg/mL with zone of inhibition 12 mm and above with methanol extract showing highest activity against salmonella typhi (17 mm) were as the two compounds showed insignificant activities against S. aureus with zone of inhibition 8 mm each. These results support some of the uses of the plant in folk medicine.

***Key words**: Syzygium guineense, phytochemical analysis, antibacterial activity, steroids.*

1. INTRODUCTION

1.1 Background

Plants have formed the basis for traditional medicines that have existed for thousands of years and continue to provide mankind with remedies. According to the WHO, over 80% of the world's population in developing countries still depends on traditional medicine for their primary health care in one way or another [1]. Traditional medicine is used throughout the world as it is dependent on locally available plants, which are easily accessible, and capitalizes on traditional wisdom-repository of knowledge, simple to use and affordable [2]. In Ethiopia, the use of traditional medicinal plants is widely practiced. The wide spread use of traditional medicine in Ethiopia could be attributed to cultural acceptability, efficacy against certain type of diseases, physical accessibility and economic affordability as compared to modern medicines [3]. The WHO also supports the use of traditional medicines as it is believed that herbal medicines are naturally superior to synthetic drugs and they are proven to be efficacious and safe [1]. The therapeutic potential, including the antioxidant, antimicrobial, and anticarcinogenic properties of plants, is due to the presence of phytochemical constituents [4] which provide definite physiological action on the human body when administered.

Phytochemicals are naturally occurring in leaves, fruits, vegetables and roots of these plants, and used for defense mechanism and protect the plants from various diseases. They could be categorized as primary and secondary compounds. For instance, chlorophyll, proteins and common sugars are included in primary constituents whereas classes of compounds such as terpenoids, alkaloids, flavonoids, saponins, tannins, steroids, anthraquinonones, phenolic compounds, and so on are considered as secondary metabolites [5].

Secondary metabolites have very little known functions in plants. Medicinal properties of plants are due to the combinations of these secondary metabolites that are synthesized and deposited in specific or in all parts of the plant. These medicinal properties are specific in a plant family, genus and species proving the fact that combinations of secondary metabolites are distinct between plant taxa [6]. Composition of secondary metabolites varies between (i)

tissues such that the bark, heartwood, roots, branch basses and wound tissues have higher concentration, (ii) species and (iii) seasons [7].

Over the past few years, there has been a tremendous resurgence of interest in medicinal plants [8]. This revival might be attributed to several driving factors such as rise in population, insufficient supply of modern drugs in certain parts of the world, prohibitive cost of treatments for common ailments, side effects of several allopathic drugs in current usage as well as development of resistance to currently used drugs [9]. Consequently, the search for scientific information about efficacy and safety of plants used in alternative medicine is a continuous process. Hence, exploitation of medicinal plants for bioactive compounds is of great potential and could be an imperative source of providing new vistas for novel drug discovery and development [10]. Therefore, isolation of natural products from higher plants, marine organisms and microorganisms is therefore still urgently needed.

Syzygium guineense is a member of the family *Myrtaceae.* The plant is found widespread in the regions of Australia, Asia and Africa including Ethiopia [11]. Myrtaceae is a plant family widely used in folk medicine in different countries in which *Syzygium* is among its most important genera [12]. The plant is being used in traditional medicine by many Ethiopian communities. For instance, oral administration of its fruits and bark are used for treatment of dysentery and diarrhea. The hot water extract of its fresh bark is used for treatment of stomachache [13].

Other authors also reported traditional medicinal uses of roots, barks and leaves of the plant for treatment of malaria, snake bite, hemorrhoid, gonorrhea, tuberculosis, stomach ache, liver problem and expelling internal worms [14-16]. It is also included among the African plant species that are active against malaria [17].

The antibacterial activity of hydro soluble dry extracts against *Salmonella E., Shigella D., Shigella F., E. coli, Enterobacter A.* has also been reported [18]. However, to the best of our knowledge, there is no phytochemical study that has been carried out to identify the active metabolites from the root of *S. guineense*. Therefore this thesis presents the result of the research done on phytochemical and antibacterial studies of the roots of *S. guineense*.

1.2 Statement of the problem

A number of researchers reported biological activity of the crude extracts from different parts of the genus as well as the pure isolated compounds. However, there are limited phytochemical studies that have been carried out to identify the active metabolites from the roots of *S. guineense*. Thus, in this research work, an attempt was made to isolate chemical constituents of the roots of the plant and examine antibacterial activity of the crude extracts as well as isolated compounds. To the best of our knowledge, there was no prior publication that comes out from the phytochemistry perspective on the root extracts of *S. guineense* from Ethiopian flora.

1.3. Significance of the study

The emergence and prevalence of diseases such as HIV/AIDS and cancer, and resistance of pathogenic organisms to known agents, still drives the needs to explore the rich world of plants with a view to find new lead compounds that can cure these diseases that are threatening mankind. Clinical observations on traditional remedies are possible and useful. It is therefore, important to investigate each medicinal plant to ascertain its full potentials [19]. This study focuses on isolation, structural elucidation of compounds from root extract of S. *guineense* and evaluation of anti bacterial activities of the root extracts and compounds isolated from the extracts. Therefore, understanding the structure of the natural product from the root extract of *S. guineense* coupled with examining the antibacterial activity have much significance to validate the traditional use of the plant.

1.4 Objectives

1.4.1 General objective

The overall objective of this study is to extract, isolate, characterize chemical constituents from roots of *S. guineense* and evaluate for antibacterial activities.

1.4.2 Specific objectives

- To carry out solvent extraction of the roots using dichloromethane: methanol (1:1) and methanol.
- To carry out phytochemical screening of the crude extracts.
- To isolate the chemical constituents from root extract using silica gel column chromatography.
- To characterize structures of the isolated compounds using different spectroscopic techniques such as IR and 1-D NMR (^{1}H-NMR and ^{13}C-NMR) and DEPT-135.
- To evaluate the antibacterial effect of the crude root extracts and compounds isolated from the extracts against *staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi*.

2. LITERATURE REVIEW

2.1 Botanical descriptions of *Syzygium guineense*

Syzygium is a genus of flowering plants that belongs to the family Myrtaceae. This genus comprises about 1200 species, and has a native range that extends from Africa to Asia. Members of the genus such as *Syzygium cumini* [20], *Syzygium jambos* [21] *Syzygium cordatum* [22], *Syzygium aromaticum* [23] *and Syzygium fruticosum* [24] have been shown to exhibit antimalarial, anti-diabetic, antibacterial, central nervous system depression antioxidant, anticancer properties. In addition, *Syzygium aromaticum, Syzygium cordatum* root and stem bark decoctions are traditionally used for treatment of malaria [25]. Another species that belongs to the family Myrtaceae and common in different parts of Ethiopia is *Syzygium guineense.* It has several local names. For instance, "water berry" in English, "Baddessa" in Afaan Oromo, "Dok'ma" in Amharic, "Ocha" in Gamo and "Karava" in Gurage. *S. guineense* is a medium-sized to large evergreen tree and usually grows up to 8-15m high (Figure 1) [26]. The bark is smooth and greyish-white to grey-brown in young trees, turning rough, flaky, creamy, light grey, dark brown or black in older trees. The leaf is broadly lanceolate, opposite, entire to the branch. The fruit is ellipsoid drupe, purplish in color and edible with higher nutritional value [27, 28]. *S. guineense* is native to Africa. It is found widely and abundantly in Ethiopia, Eritrea, Kenya, Lesotho, Mozambique, Namibia, Senegal, Somalia, South Africa, Swaziland, Tanzania, Uganda, Zambia, Botswana and Zimbabwe.

Figure 1 A typical plant of *Syzygium guineense* (Photo taken from Mendi town, West Wollega Zone, Oromia, Ethiopia, December 2017 by Ayele Bekele)

2.2 General uses of *Syzygium guineense*

The tree is used as a fire wood and shade in coffee plantations in Ethiopia. The plant is found in the altitude range 2,300 - 2,700 m. *S. guineense* has been used to alleviate symptoms of different diseases in some parts of Africa. It is a flowering plant native to the wooded savannah and tropical forests of Africa. Also known as 'water berry', the plant is used locally as charcoal, timber, food, medicine, fodder, bee forage and dyes [29].

2.3 Ethno Pharmacological Information of *S. guineense*

Traditionally, many morphological parts of this plant have been utilized in the management of various ailments in many Ethiopian communities. For example, oral administration of its fruits and bark are used for the treatment of dysentery and diarrhea and infusion prepared from its leaves, fruits or bark is used for treatment of hypertension [3]. For instance, a mixture of water and powder made from the bark and roots of the plant when administered act as a purgative [30].

Bark decoctions of *S. guineense* is also used traditionally to treat stomachache, diarrhea and malaria in many counties. Stem bark of *S. guineense* is used traditionally against HIV/AIDS opportunistic infections such as herpes zoster and chronic diarrhea in West Africa [32]. Root and stem bark of the plant also used to treat internal parasite, stomachache and urinary problem in Ethiopia [14]. *S. guineense* is also one of the medicinal plants utilized by traditional practitioners to control ectoparasites infection in livestock. On top of this, *S. guineense* is one of the African plant species that are traditionally used against malaria. For instance, the decoction of the stem bark and leaf of *S. guineense* is commonly used by traditional healers' in Ethiopia [31, 33], south Uganda [34], D. R. Congo [35] and Guinea [36] for treatment of malaria.

2.4 Biological activities of *S. guineense*

Biological studies revealed that *S. guineense* has a wide range of activities including spasmolytic, antidiarrheal, antibacterial, anti-inflammatory and analgesic activities. The leave of *S. guineense* is also used as antiseptic against infection [37]. Triterpenes (Arjunolic and Asiatic acids) isolated from the plant have been reported to be biologically active against bacteria (*Escherichia coli, Bacillus subtilis* and *Shigella sonnei*) [38].

Its dried twig, stem bark and fruits have also prominent spasmolytic, antitransit and against castor oil-induced antidiarrheal properties [39]. In addition, the hydro-alcoholic extract of the leaves of *S. guineense* has been reported to have antihypertensive activity [40]. Ethanolic extract of the leaves of *S. guineense* has also been shown to possess anti-inflammatory and analgesic properties [41]. In addition, the plant is endowed with compounds with immunomodulatory activities [42]. The extract of stem barks of *S. guineense* also showed strong antioxidant properties and act as free radical inhibitors or scavengers [43].

A literature report revealed that a dried aqueous decoction of *S. guineense* showed activity against strains of *Salmonella E*., *Shigella D*., *Shigella F*., *E. coli* and *Enterobacter A*.[44]. Malele et. al (1997) reported that a methanol extract of *S. guineense* bark inhibited intrinsic contractions of rabbit isolated ileum [45]. Amadou D. in 2005 showed antioxidant and anti inflammatory activities of an aqueous decoction of the leaves of *S. guineense* [46]. Oyewale in 2007 showed the antimicrobial effects of secondary metabolites identified from *S. guineense* from Nigeria, and cytotoxicity tests carried out on plant extract showed activities against the following pathogens: *Staphylococcus aureus, Escherichia coli, Candida albican Salmonella typhi, Klebsiella pneumonia and Pseudomonas aeruginosa* [47]. In 2008, Mpiana et.al reported the antisickling activity, *in vitro*, of the leaves of *S. guineense* collected in DRC [48].

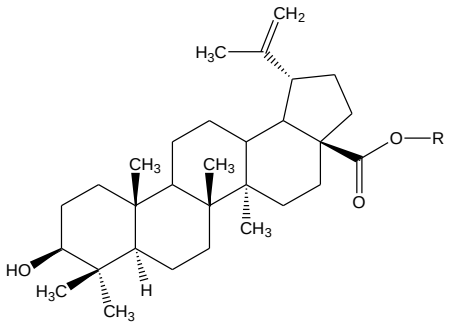
Reports also revealed insecticidal activity of leaves of *S. guineense* against *Melophagus ovinus* (an external parasite of sheep belonging to the family *Hippoboscidae*) [49]. In another study, the root bark showed antimycobacterial activity by minimum inhibitory concentration within the range of 800 μg/ml to 2000 μg/ml [50].

2.5 Phytochemistry of *S. guineense*

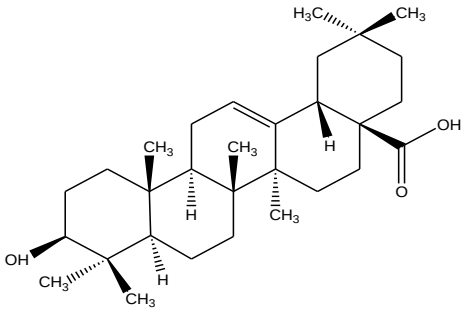
Phytochemically, several members of this family mainly possess secondary metabolites such as flavonoids, tannins, other phenolic derivatives and terpenoids [51]. Currently, there is an increased interest in terpenoids for antibacterial, antineoplastic, and other pharmaceutical functions [52]. Phytochemical analysis of the leaves of S. guineense showed the presence of flavonoids, tannins, saponins, carbohydrate, alkaloids and cardiac glycosides [53], arabinogalactan polysaccharides that possessed immunological activities [54]. Phytochemical studies carried out on the leaves of *S. guineense* from Mali and from Nigeria was carried out and

showed the presence of some secondary metabolites such as alkaloids, tannins, sterols, triterpenes, anthocyanins, leucoanthocyanins, saponins, and flavonoids [46]. Other secondary metabolites present in *S. guineense* implicated for their antimalarial activity include: terpenoids [55], phenols [56], anthraquinones [57], and flavonoids [58]. A recent study demonstrated that leaves, stem bark and roots of S. guineense have antioxidant properties and are rich in polyphenols [59]. The chemical composition of essential oil from *S. guineense* was also investigated. Caryophyllene oxide (7%), δ-cadinene (7.5%), viridiflorol (7.5%), *epi*-α- cadinol (9.8%), α-cadinol (12.7%), *cis*-calamenen-10-ol (14%), citronellyl pentanoate (15.2%), β-caryophyllene (20.1%) and α-humulene (39.5%) are essential oils found abundantly in dried leaves of *S. guineense* [60]. Djoukeng *et al*.[32] reported isolation of ten triterpenes from the leaf extracts of *S. guineense* namely betulinic acid (**1**), oleanolic acid (**2**) , a mixture of 2α-hydroxyoleanolic acid (**3**), 2α -hydroxyursolic acid (**4**), arjunolic acid (**5**) and asiatic acid (**6**), a mixture of terminolic acid (**7**), and 6-hydroxyasiatic acid (**8**), and a mixture of arjunolic acid 28- β -glucopyranosyl ester (**9**) and asiatic acid 28- β -glucopyranosyl ester (**10**).

Oladosu *et. al.* (**2017**) reported isolation of 3-β-hydroxylupane-type isoprenoids: betulinic acid methylenediol ester (**1'**) and betulinic acid (**1**) (Figure 2) from chloroform extract of stem bark of Syzygium guineense [61]. Bihon *et. al.*(**2018**) reported isolation of 2,3,23-trihydroxyl methyl oleanate (**16**) (Figure 2) from dichloromethane/methanol (1:1) extract of roots of *Syzygium guineense* [62]. Abok and Manul (**2016**) reported detection of 12 compounds from n-hexane extract of leaves of S. guineense using TLC and GC- MS analysis. Some of the compounds were 1-ethyl-2-methylbenzene (**11**), Ylangene (**12**), decahydro-4a-methyl-1-methylene-7-(1-methylethynyl)-naphthalene (γ-muurolone) (**13**), 4-dimethyl-7-(1-methylethenyl)azulene (**14**), caryophyllene oxide (**15**) [63] Phytochemical study of the leaves of *S. guineense* from Mali and from Nigeria was carried out and showed the presence of some secondary metabolites such as alkaloids, tannins, sterols, triterpenes, anthocyanins, leucoanthocyanins, saponins and flavonoids [47].



(1) R = H; (**1'**= CH_2-OH)



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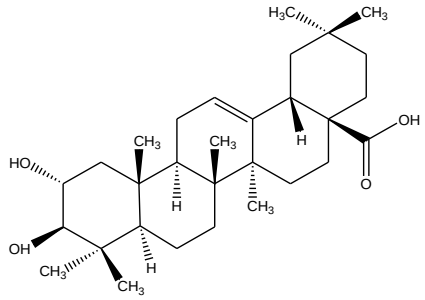

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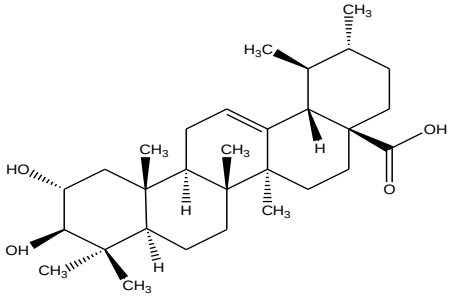

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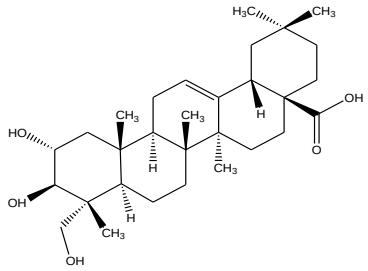

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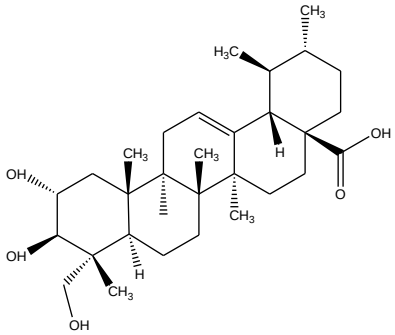

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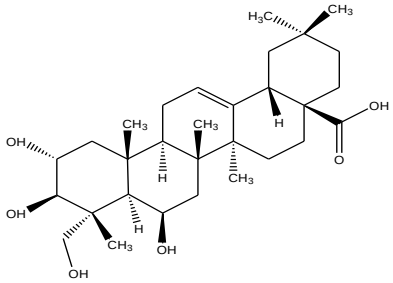

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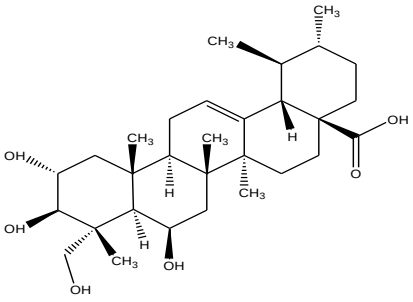

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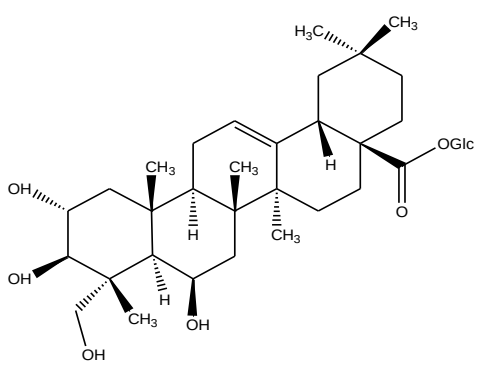

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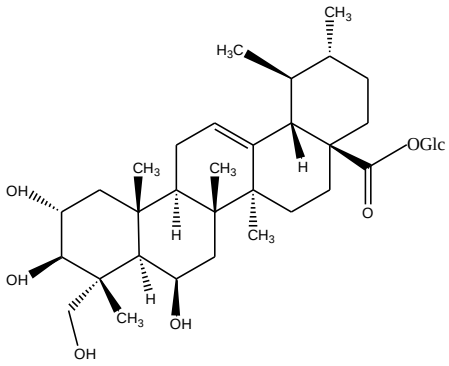

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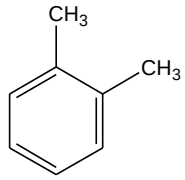

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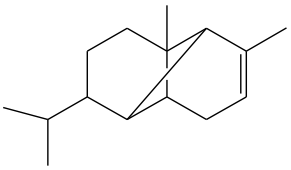

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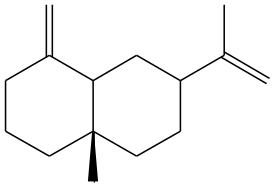

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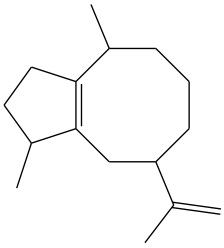

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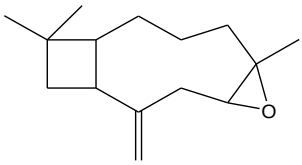

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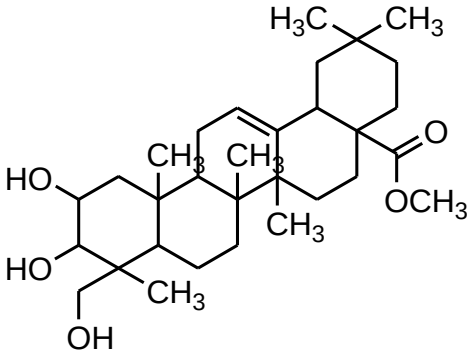

(**16**)

Figure 2. The chemical structures of compounds (1-16) isolated from *Syzygium guineense*

3. MATERIALS AND METHODS

3.1 General Experimental Procedures

Silica gel Column chromatography was performed using silica gel (200-400 mesh) Merck. Analytical Thin Layer Chromatograms were conducted by using 0.25 mm thick layer of silica gel GF_{254} (Merck, Germany) coated on aluminum plate. Spots of compounds on TLC were detected using UV-254nm or dipping in vanillin-H_2SO_4 and heating for a few minutes. Infrared spectra were obtained on Perkin- Elmer 65FT ((IRvmax, KBr (4000-400) cm^-) infrared spectrometer using KBr pellets. NMR spectra of the compounds were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ and DMSO operating at 400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR and DEPT-135 with TMS as internal standard. The glassware used was cleaned using soap and distilled water, rinsed with acetone or ethanol, and finally dried in an electric oven at 100 ^oC.

3.2 Reagents and chemicals

The solvent Chemicals such as: Methanol (CH_3OH, 99.8%) (Loba Chemie Pvt. Ltd., India), Dichloromethane (DCM, 99.5%) (Loba Chemie Pvt. Ltd., India), n-hexane ($C_6H_{12,}$ 99%) (Loba Chemie Pvt. Ltd., India), ethyl acetate ($C_4H_8O_2$, 99.5) (ATICO Medical Pvt. Ltd., India) were purchased from Alchem Import p.l.c, Addis Ababa, Ethiopia and used for extraction. Other chemicals and reagents that were used to test the preliminary phytochemical constituents, for detection of compounds on TLC plates and to evaluate the anti bacterial activities of the roots extracts of *Syzygium guineense* were: sulphuric acid, sodium hydroxid, hydrochloric acid, ammonia, distilled water, acetone, ferric chloride, acetic acid, chloramphenicol, sodium carbonate, ethanol and silica gel. All these chemicals were of analytical grade.

3.3 Apparatus and instruments

TLC plates, TLC chamber, rotary evaporatory (R1001-VN (-V1,E,G), Zhengzhou Great Wall S & I TCo.LTD, China), sprayer, reagent bottles, capillary tubes, round bottom flasks, UV lamp, beakers (different sizes), separatory funnels, Watmann No.1, filter papers, dropper, ruler, pencil, stirrer, drying oven, measuring cylinders (different sizes), digital electronic balances (ESJ200-4,

Zhengzhou Nanbei Instrument Equipment Co., Ltd., China), conical flasks (different sizes), and chromatographic column.

3.4 Plant material collection, identification and preparation

The fresh roots of *S. guineense* (Figure 3) plant were collected from Mendi town, West Wollega Zone, Oromia, Ethiopia, which is 565 km west of Addis Ababa on December 20, 2017. The plant specimens were brought to the National Herbarium (ETH) and identified by Melaku Wendafrash, Addis Ababa University. The plant is then identified by comparing with the authenticated specimens housed at ETH and by referring to published works on flora of Ethiopia and Eretria. The collected roots of the plant were thoroughly washed using tap water to remove dirtiness and were dried in an open air in the shade at the laboratory of Menesibu Preparatory School (Mendi Town); protected from direct exposure to sun light. The dried plant roots were finely grounded using local wooden grinding mill and made ready for extraction.

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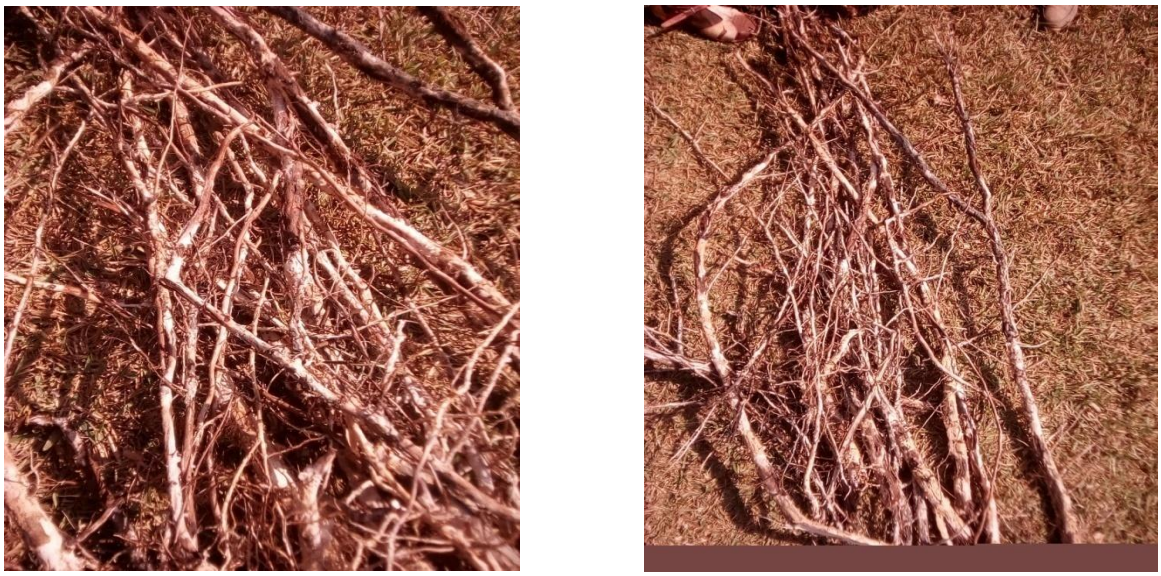


Figure 3 The fresh roots of *Syzygium guineense* (Photo taken from Mendi town, West Wollega Zone, Oromia, Ethiopia, December 2017 by Ayele Bekele)

3.4 Experimental procedures

3.4.1 Extraction

The pulverized roots of *S. guineense* (500 g) were soaked in dichloromethane/methanol (1:1) (4 L) and extracted at room temperature using maceration technique for 3 days, with continuous shaking. After 3 days the solution was filtered by Whatman (no.1) filter paper. The marc obtained from the filtration was subjected for further extraction by methanol (2.5 L) for 3 days. The filtered solutions were concentrated under reduced pressure (40 ^{o}C) using rotary evaporator to give their corresponding crude extract. The resulting semidried extract of each solvent was weighted and stored in refrigerator at 4 ^{o}C until needed for the further analysis; microbial assay and TLC analysis. The extraction yields obtained were calculated using the formula;

Percent yield of the crud extract (crude extract yield) = (weight of dry extract/weight of dry material) x100

3.4.2 Isolation of compounds

The DCM: MeOH extract (5.0 g) was adsorbed on equal amount of silica gel and subjected to silica gel (150.0 g) column chromatography eluted with increasing gradient of ethyl acetate in *n*-hexane.

Table 1. Solvent system and the fractions collected from DCM: MeOH crude extract of the root of *S. guineense*

Fraction number	Volume (mL) of each fraction	Eluent	Ratio	Volume (mL)
1-2	50	*n*-hexane	100%	100
3-4	50	*n*-hexane: EtOAc	9:1	100
5-7	50	*n*-hexane: EtOAc	8:2	150
8-21	50	*n*-hexane: EtOAc	7:3	700
22-26	50	*n*-hexane: EtOAc	6.5:3.5	250
27-28	50	*n*-hexane: EtOAc	6.4:3.6	100
29-36	50	*n*-hexane: EtOAc	6:4	350
37-44	50	*n*-hexane: EtOAc	5.8:4.2	350
45-50	50	*n*-hexane: EtOAc	5.5:4.5	300
51-56	50	*n*-hexane: EtOAc	50:50	200
57-60	50	*n*-hexane: EtOAc	4.5:5.5	300
61-66	50	*n*-hexane: EtOAc	40:60	300
67-70	50	*n*-hexane: EtOAc	30:70	200
71-76	50	*n*-hexane: EtOAc	20:80	300
77-80	50	*n*-hexane: EtOAc	10:90	200
81-84	50	*n*-hexane: EtOAc	100%	200

For DCM: MeOH extract, a total of 84 fractions were collected. Fractions 1- 4 showed no visible spots on TLC. Fractions 5-7 showed two spots on TLC which was visualized under UV254nm with n-hexane: EtOAc (7:3) as a mobile phase. Fraction 8-9 showed a clear single spot on TLC having a retention factor (R_f) of 0.528 with n-hexane: EtOAc (7:3) as a mobile phase. The crystalline solids of fractions 8 and 9 were soluble in n-hexane; showed the compound was non polar compound. Fractions 10-12 sowed two spots having different R_f on TLC with the same solvent system for eluent as in fraction 8-9 and visualized under UV254nm. Fractions 13-19 showed three spots having different R_f on TLC with *n*-hexane: EtOAc (7:3) as a mobile phase. Fractions 27-39 all showed more than two spots having different R_f on TLC using n-hexane:

EtOAc (5:5) as eluent. Fractions 40-48 form more than two spots which were not clearly visualized on TLC under UV254nm with *n*-hexane:

EtOAc (6:4) as mobile phase rather they form streaky spots. Fractions 49-61, 70-71 also showed more than two spots which were visualized on TLC under UV254/365 nm with n-hexane: EtOAc (4:6), and (8:2), respectively, as a mobile phase. Fractions 72-76 each showed single spot on TLC with EtOAc: MeOH (9:1) as mobile phase visualized under UV254. Fractions 77-84 showed two major spots which were streaky on TLC with EtOAc: MeOH (9:1) as mobile phase visualized under UV254. Fractions 72-76 (180 mg) which showed similar TLC, with one spot, were combined to afford compound 5.

Table 1 Solvent system and the fractions collected from MeOH crude extract of the root of *S. guineense*

Fractions collected	Eluent	Ratio	Volume (mL)	Spot on TLC	Fractions collected	Eluent	Ratio	Volume (mL)	Spot on TLC
1-2	*n*-hexane: EtOAc	9.5:5	100	-	62-63	*n*-hexane: EtOAc	3.5:6.5	100	2
3	*n*-hexane: EtOAc	9.4:6	100	-	64-67	*n*-hexane: EtOAc	3:7	200	2
4-5	*n*-hexane: EtOAc	9.3:7	100	-	68	*n*-hexane: EtOAc	2.5:7.5	100	2
6	*n*-hexane: EtOAc	9.2:8	100	-	69-72	*n*-hexane: EtOAc	2:8	200	2
7	*n*-hexane: EtOAc	9.1:9	100	3	73-76	*n*-hexane: EtOAc	1.5-7.5	200	2
8-10	*n*-hexane: EtOAc	9:1	150	3	77-81	*n*-hexane: EtOAc	1:9	250	2
11	*n*-hexane: EtOAc	8.9:1.1	100	3	82	EtOAc	100%	100	2
12-14	*n*-hexane: EtOAc	8.8:1.2	100	3	83-91	DCM: MeOH	9.5:5	400	2
15	*n*-hexane: EtOAc	8.7:1.3	100	2	92-97	DCM: MeOH	9:1	300	3
16	*n*-hexane: EtOAc	8.5:1.5	100	2	98-99	DCM: MeOH	8.5:1.5	100	3
17	*n*-hexane: EtOAc	8.3:1.7	100	4	100-101	DCM: MeOH	8:2	100	3
18	*n*-hexane: EtOAc	8:2	100	4	102-103	DCM: MeOH	7.5:2.5	100	2
19	*n*-hexane: EtOAc	7.8:2.2	100	4	104	DCM: MeOH	7.5:2.5	50	2
20-21	*n*-hexane: EtOAc	7:3	200	4	105-107	DCM: MeOH	7:3	150	5
22-24	*n*-hexane: EtOAc	6.8:3.2	200	2	108-113	DCM: MeOH	6.5:3.5	300	5
25-26	*n*-hexane: EtOAc	6.5:3.5	100	2	114-115	DCM: MeOH	6:4	100	3
27-29	*n*-hexane: EtOAc	6.3:3.7	100	2	116	DCM: MeOH	55:45	100	3
30-37	*n*-hexane: EtOAc	6.0:4.0	400	2	117	DCM: MeOH	5:5	100	-
38	*n*-hexane: EtOAc	5.8:4.2	100	2	118	DCM: MeOH	4:6	10	-
39	*n*-hexane: EtOAc	5.6:44	100	2	119-122	DCM: MeOH	3.5:6.5	200	-
40-42	*n*-hexane: EtOAc	5.5:4.5	300	2	123	DCM: MeOH	3:7	100	-
43	*n*-hexane: EtOAc	5.3:4.7	100	2					
44-48	*n*- hexane: EtOAc	5.2: 4.8	200	2					
49-51	*n*-hexane: EtOAc	5:5	300	2					
52-55	*n*-hexane: EtOAc	4.7:5.3	200	2					
56	*n*-hexane: EtOAc	4.5:5.5	100	2					
57-61	*n*-hexane: EtOAc	4.0:6.0	250	2					

For methanol crude extract column chromatographic separation was performed. The MeOH extract (8.0 g), was a light brown colored solid. It was first adsorbed on to an equal amount of silica gel and was subjected to column chromatography over silica gel (200-400 mesh, 150.0 g) using n-hexane for packing. The column was eluted with solvent system of gradually increasing polarity using n-hexane: Ethyl acetate (EtOAc) (solvent ratios: 100:0 to 0:100 %) and then with DCM: MeOH (9.5: 0.5 to 3:7). The collected fractions were monitored on the TLC plate (silica gel 60 F254 aluminum backing from Merck, Germany).

A total of 123 fractions were collected. The fractions 22-27 (181 mg) which showed similar TLC profile with two spots, were combined and re-chromatographed over silica gel (25 g) using isocratic solvent system *n*-hexane: EtOAc (6:4) as eluent to furnish 12 fractions each 10 mL.

Fractions (9-11, 35 mg, red colored), showed similar TLC profile with one spot were combined and gave the second compound, derivative of lupeol (**18**).

Fractions 28-34 (40.9 mg) which showed similar TLC profile with two spots were combined and re chromatographed over silica gel (25 g) using isocratic solvent system *n*-hexan: EtOAc (6:4) as eluent to furnish 22 fractions each 5 mL. Fractions (17-21, 2.3 mg, whit, R_f= 0.38) showed one colored spot on TLC.

Fractions 100-102 which showed similar TLC profile were also combined and re chromatographed over silica gel (25 g) using isocratic solvent system DCM: MeOH (8:2) as eluent to furnish 10 fractions each 5 mL. Fractions (7-9, 4.5 mg, white, R_f= 0.48) showed one colored spot on TLC with solvent system of DCM: MeOH (7:3) as eluent. The scheme of the extraction, fractionation and isolation procedure are shown below (Figure 4).

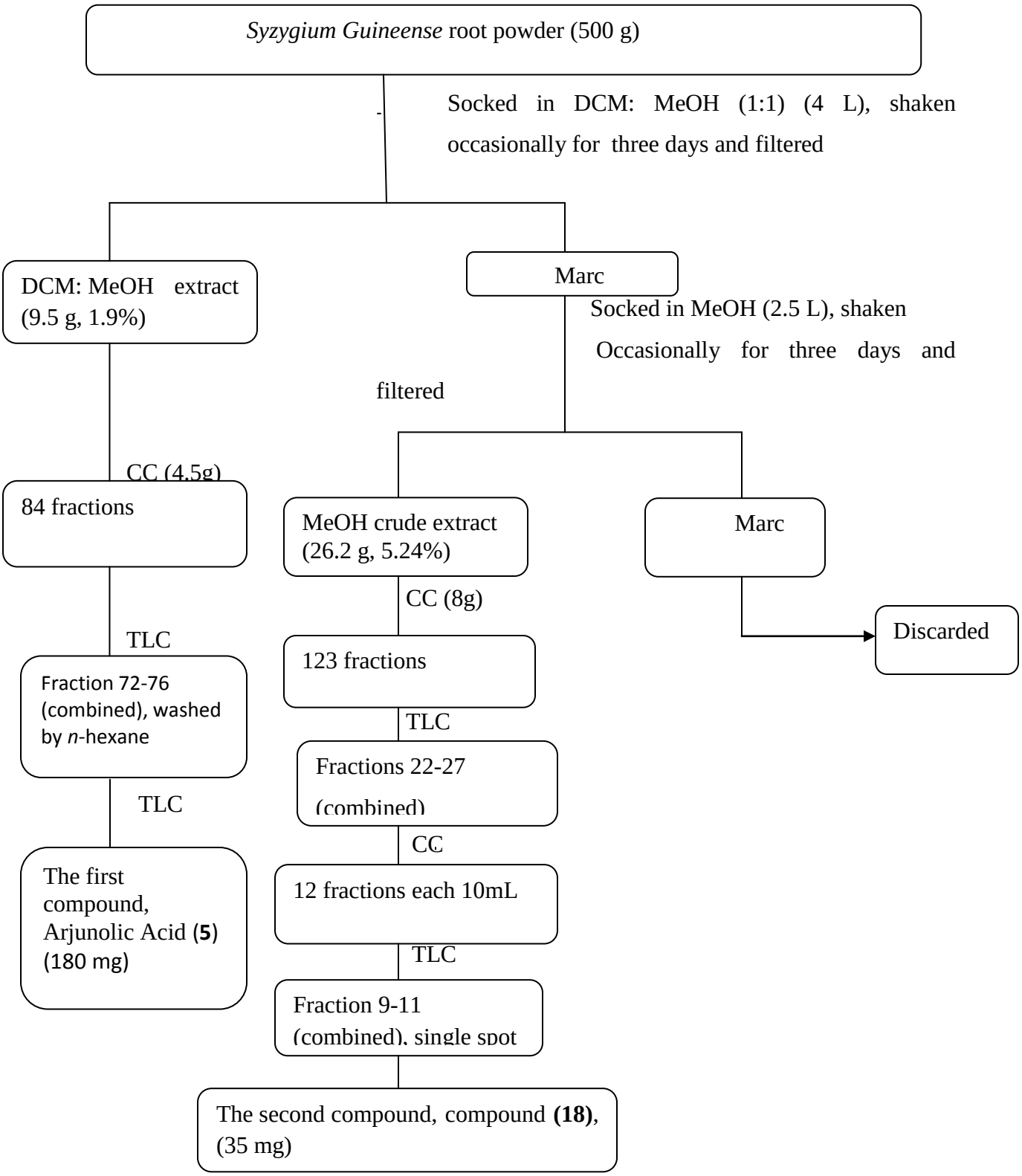


Figure 4. The general scheme of extraction and isolation of compounds from the root of *Syzygium guineense*.

3.4.3 Preliminary phytochemical screening tests

The preliminary phytochemical screening tests were carried out for each crude extract (Dichloromethane/methanol and methanol extracts) using standard procedures reported in literature [64-66] to identify the constituents (secondary metabolites) such as alkaloids, flavonoids, phenols, glycosides, saponins, terpenoids, tannins and saponins). Phytochemical screening was done qualitatively using color forming and precipitating chemical reagents on the crude extracts of the plant (Table 4).

3.4.3.1 Test for alkaloids

Wagner's test

To each crude extract (3 mL) dissolved in methanol, 1 mL of 1% HCl was added in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 1 mL of each of the filtrate was treated with Wagner's reagent (I_2 and KI in 100 mL water) and formation of reddish brown precipitate indicates the presence of alkaloid [64].

3.4.3.2 Test for flavonoids

Alkaline Reagent Test

3mL of each of the extract dissolved in methanol was treated with 3 drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids [64].

3.4.3.3 Test for phenols

2 mL of each of the extracts dissolved by water was treated with a few drops of 2% of $FeCl_3$. Formation of green or bluish black color indicated the presence of phenols [65].

3.4.3.4. Test for glycosides

Keller-Killani test

Each extract (0.5 gm) weighed placed in to test tube were each dissolved in 1mL of glacial acetic acid containing traces of ferric chloride. Sulphuric acid (2 mL) was then added carefully drop wise and shaken gently. A color change from orange to a reddish-brown color at the interface of the two layers indicated the presence of glycosides [65].

3.4.3.5. Test for terpenoids

Salkowski test: The extracts (0.5 g) each were shaken with chloroform (2 mL) in the test tube. Concentrated sulfuric acid (3 mL) was then added to each of the test tubes along the side by using dropper. A reddish brown coloration at the interface confirmed the presence of terpenoids [65].

3.4.3.6. Test for tannins

0.5 g of each of the crude extract was boiled in 20 mL of distilled water in a test tube and then filtered. A few drops of 0.1 % $FeCl_3$ was added to give a brownish green or blue black coloration confirmed the presence of tannins [65].

3.4.3.7. Test for saponins

Froth test: 0.2 g of each extract was dissolved in 5 mL of distilled water in a test tube, shaken and heated to boil on a water bath. Formation of layer of foam indicated the presence of saponins [66]

3.4.3.8. Tests for steroids

Two milliliter of acetic anhydride was added to each of the extract (2 mL) that was dissolved in methanol. 2 mL of sulphuric acid was then added slowly by the sides of the test tube containing the mixture. Formation of a blue-green ring indicates the presence of steroids [65].

3.4.4 Spectroscopic analysis

Structures of the isolated compounds were elucidated based on spectroscopic methods such as IR, NMR (^{1}H-NMR and ^{13}C-NMR) and DEPT-135 spectra in deuterated chloroform ($CDCl_3$), DMSO and MeOH as a solvent deemed necessary with tetra methyl silane as internal standard and comparing the result with reported value from literature.

3.5 Antibacterial testing

3.5.1 Bacterial culture

One gram-positive *Staphylococcus aureus* (ATCC No. 25923) and three gram-negative *Escherichia coli* (ATCC No. 25922), *Klebsiella pneumoniae* (ATCC No. 700603), and *Salmonella typhi* (13311) isolated from stool specimens were identified according to routine cultural properties and biochemical tests. A few colonies from the overnight culture of Eosin Methylene Blue (EMB) agar was transferred in to approximately 4-5 ml Tripticase soy broth (TSB) medium. The broth was incubated at 37 ^{o}C for 3-4 hours and the turbidity of suspension was adjusted to that of a 0.5 Mc Farland barium sulfate standard [67].

3.5.2 Evaluating antibacterial activity of the root extracts of *S. guineense*

Dichloromethane: methanol (1:1) and methanol extracts and the isolated pure compounds of the root of *S. guineense* were evaluated for anti bacterial assay by using agar-well diffusion method described by [50]. The bacterial strains were obtained from the American Type Culture Collection (ATCC) and the antibacterial activities of all samples were tested against one gram positive *staphylococcus aureus* and three gram- negative *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi* using Muller Hinton Agar (MHA) medium. All the microbial were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Standard antibiotic Gentamicin of concentration 1 mg/mL was used as positive control against bacteria. DMSO was used as a negative control.

Media Preparation

Muller Hinton Agar medium was prepared by dissolving the solid media in 10 mL distilled water. The dissolved medium was then autoclaved at 120 ^{o}C for 15 minutes. After autoclaving, the media was cooled to about 50 ^{o}C. The medium was then poured to sterile Petri dishes, allowed to solidify and used for the disc diffusion antibacterial activity assay [67].

Inoculation and Incubation

The test bacterial species were transferred from the stock cultures and streaked on Muller Hinton plates. Well-separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved Mullar Hinton agar and mixed by gently swirling the flasks. 0.1 g of each of the extracts and 2 mg of each of the isolated compounds were dissolved in 1ml of DMSO separately and 200 μg of each of the extracts and 100 μg/mL of each of the compounds were prepared. Wells of 6mm diameter were made in the culture medium using sterile cork borers and 10 μL of 0.5 mg/ml and 20 μL of 1 mg/mL concentration of the sample were placed in the wells using micropipette and the plates were kept for incubation at 37 ^{o}C for 24 hrs [67]. Control of each bacterial strain before sample action was at 6 mm. Each of the prepared test solutions was taken to test its sensitivity towards the selected bacteria. The antibacterial activity, indicated by an inhibition zone surrounding the well, was assessed by measuring the inhibition zone diameters in millimeters formed around the well against each test organism at different concentrations and recorded if the zone of inhibition was greater than 6 mm.

4. RESULTS AND DISCUSSION

4.1 Extract yield.

The solvents, DCM: MeOH (1:1) and MeOH were successively used to extract phytochemicals from the roots of *S. guineense*. The percentage (extraction) yields reflect the amount or yield of extractable components relative to the weight of dry plant material. It is a factor of several variables including the extraction solvent system, nature of the sample, method of extraction, time of extraction, temperature and pressure. Different solvents, depending on their selectivity and polarity, extract varying amounts of different groups of phytochemicals.

Table 3. Extracts yield of the roots of *S. guineense*

Extract	Color of the crude extract	Weight of the Extract	% yield
DCM: MeOH(1:1)	Dark brown	9.5	1.90
MeOH	Brown	26.2	5.24

As shown in Table 3, total yield of the extract from the roots the plant was about 7.1%. As it has been shown, the percentage yield increased with increasing polarity. The extracts obtained increased with polarity of the solvent likely implying that the plant contains more polar compounds as compared to the non polar compounds.

4.2. Phytochemical Screening of the roots of *S. guineense*

Phytochemical screening tests were made for each crude extract of Dichloromethane/methanol and methanol using standard procedures reported in literature [64-66]. Therefore, the result showed that the chemical compounds such as alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, steroids and saponins were present in both DCM: MeOH and MeOH extracts. This observation was consistent with previous reports mentioning that different parts of *S. guineense* are rich in secondary metabolites that could be attributed to the medicinal uses of the plant discussed in Section 2.5 [47, 55, 56, 58, and 59].

Table 4. The phytochemical screening test results of root extracts of *Syzygium guineense*

Phytochemicals	**Dichloromethane: methanol (1:1) extract**	**Methanol extract**
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Glycosides	+	+
Terpenoids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+

Note: (+) indicates the presence of particular metabolite.

This indicates that most of the compounds found in this plant extracts are polar organic compounds and shows that the plant constituents might have high level of medicinal values. This could be responsible to the medicinal properties of the plant in treating different ailments.

4.3 Structural elucidation of isolated compounds

In this work two compounds were isolated from the extracts of roots of *S. guineense*. Described herein is the characterization of these compounds in detail.

Compound 5

Compound **(5)** was obtained from dichloromethane: methanol (1:1) extract (Section 3.4.2) as white solid with R_f value of 0.41 in *n*-hexane: ethyl acetate (1:9) as a mobile phase. The IR spectrum (Appendix 1) showed a broad absorption around 3418 cm^{-1}, strong absorption bands at 1679 cm^{-1} and 2930 cm^{-1} suggesting the presence of hydroxyl moiety, carbonyl (C=O) and aliphatic C-H stretching vibrations, respectively. On the other hand, medium absorption band at 1038 cm^{-1} indicates the presence of C-O stretching of carboxylic acid and a band at 664 cm^{-1} indicated absorption band for olefinic C-H bending.

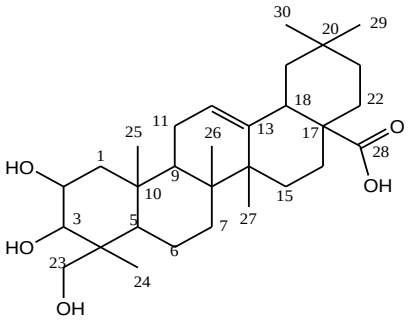
The ^{1}H-NMR spectrum (Appendix 2) showed an intense peak at δ 5.17 suggesting the presence of olefinic proton whereas the peaks at δ 3.4 and 3.0 indicate methine protons bonded to hydroxyl group bearing carbon atoms. The peak observed at δ 3.2 indicates protons on methylene group bearing hydroxyl functional group (CH_2-OH). Moreover, a peak at δ 2.74 indicates proton bonded to a carbon that is attached to olefinic carbon. Six aliphatic singlet methyl signals were observed at δ 0.74 (s), 0.87 (s), 0.92 (s), 1.05 (s), 1.20 (s) and 1.60 (s). The multiplet peaks at 0.88-2.2 indicating methine and methylene proton (20 protons) reported for terpenoids in literature (Table 5) [68, 71].

The proton decoupled ^{13}C-NMR spectrum (Appendix 3) with the aid of DEPT-135 (Appendix 4) revealed the presence of peaks at δ 67.9 and 76.0 accounted to sp^3 oxygenated methine groups. The peaks at δ 64.4 and 179.1 indicate oxygenated methylene (CH_2-OH) and carboxylic acid carbonyl group, respectively (Table 5). The ^{13}C-NMR and DEPT-135 data also confirmed the presence of olefinic carbons at δ 121.9 and 144.4, of which the later is quaternary, and ten methylene groups (one oxygenated methylene), six methyl group, six methine group and eight quaternary carbons. This suggests that the compound have a pentacyclic terpenoid with Olean-12-ene-skeleton [69]. Thus, based on the above spectral data, the compound (**5**) was found to be arjunolic acid (Table 5) [68, 70].

Table 5. ^{1}H NMR and ^{13}C NMR Spectral data of compound (**5**) and literature reported data of [69, 72] for Arjunolic acid.

Position of carbon	^{1}H-NMR δ(ppm) data of compound (11)	^{13}C NMR δ(ppm)	Literature report [65, 68]	
			^{1}H NMR δ (ppm) data of arjunolic acid [69, 72]	^{13}C- NMR δ(ppm) data of arjunolic acid [69, 72]
1	0.8-2.2 (2H, dd)	47.1	0.74-1.98 (2H, dd)	47.1
2	3.31 (1H, m)	67.9	3.31 (1H, m)	67.9
3	3.05 (1H, d)	76.0	3.04 (1H, d)	76.0
4	-	42.9	-	42.9
5	0.8-2.2 (1H, dd)	47.5	0.74-1.98 (1H, dd	47.5
6	0.8-2.2 (2H, td)	17.9	0.74-1.98 (2H, tp)	17.9
7	0.8-2.2 (2H, td)	32.6	0.74-1.98 (2H, td)	32.6
8	-	39.9	-	39.9
9	0.8-2.2 (1H, t)	47.3	0.74-1.98 (1H, t)	47.3
10	-	37.8	-	37.7
11	0.8-2.2 (2H, dd)	23.5	0.74-1.98 (2H, dd)	23.6
12	5.17 (1H,t)	121.9	5.17 (1H,brs)	121.9
13	-	144.4	-	144.4
14	-	42.2	-	42.2
15	0.8-2.2 (2H, dt)	27.7	0.74-1.98 (2H, dt)	27.6
16	0.8-2.2 (2H, td)	23.8	0.74-1.98 (2H, td)	23.8
17	-	45.9	-	45.9
18	2.74 (1H,dd)	41.9	2.74 (1H, dd)	41.9
19	0.8-2.2 (2H, dd)	46.5	0.74-1.98 (2H, t)	46.5
20	-	30.9	-	30.9
21	0.8-2.2 (2H, t)	33.8	0.74-1.98 (2H, t)	33.8
22	0.8-2.2 (2H, dd)	33.3	0.74-1.98 (2H, dd)	33.6
23	3.18 (2H,d)	64.4	3.17 (2H, d)	64.4
24	1.60 (3H, s)	14.2	1.54 (3H, s)	14.2
25	0.93 (3H, s)	17.4	0.97 (3H, s)	17.4
26	1.05 (3H, s)	17.5	1.06 (3H, s)	17.5
27	1.20 (3H, s)	26.2	1.18 (3H, s)	26.2
28	-	179.1	-	179.0
29	0.87 (3H, s)	33.3	0.87 (3H, s)	33.4
30	0.74 (3H, s)	23.9	0.74 (3H, s)	23.8
	δH(0.8-2.2 terpenoid protons)		δH (0.74- 1.98) terpenoid protons)	

Note: Carbon number 4, 8, 10, 12, 13, 14, 17, 20 and 28 are quaternary carbons



Compound (5) - Arjunolic acid

Figure 4 Structure of compound (**5**)

Described herein is the characterization of compound 18 in detail.

Compound (**18**)

Compound (**18**) was isolated from methanol extract as a reddish brown crystalline solid. On analytical TLC, the compound has R_f value of 0.8 in *n*-hexane: EtOAc (6:4) solvent system as mobile phase.

The chemical shifts of ^{1}H NMR, ^{13}C NMR and DEPT-135 were increased by 4 ppm units than normal for this compound. For instance, on the spectral data, chloroform appeared at δ 81.2 ppm instead of δ 77.2 ppm for ^{13}C NMR. Therefore the characterization was done by subtracting 4 ppm from the observed readings of the spectral values.

The ^{1}H- NMR spectrum (Appendix 5) (Table 6) revealed the presence of olefinic protons at δ 4.49 and 4.62 suggest the presence of a terminal double bond. Oxygenated sp^3 methine proton was observed at δ 3.87 (m, 1H, H-3) which is a characteristic of most steroids with hydroxyl group at C-3 position. The presence of six singlets, each integrating to three protons at δ 0.65, 0.724, 0.843, 0.866, 0.877 and 1.50 strongly suggested pentacyclic triterpenoids. This was in agreement to the lupeol type structure of a triterpenoid. The ^{13}C - NMR spectrum (Table 6) revealed the presence of twenty nine carbon signals which is a characteristic feature of triterpenes. The ^{13}C NMR (Appendix 5) and DEPT-135 spectra (Appendix 6) displayed the presence of six methyl carbons signals at δ 14.6, 15.2, 15.74, 15.9, 19.1, and 27.73 in agreement with pentacyclic triterpenoids.

The ^{13}C and DEPT-135 spectrum also showed ten methylene carbon signals at δ 18.19, 20.79, 25.45, 26.86, 29.57, 30.45, 32.21, 34.23, 37.1 and 38.67. Presence of five methine carbons (δ 46.91, 49.1, 50.5, 55.3 and 38.23), two olefinic carbons (δ 150.9 and 109.4), of which one is quaternary, and additional quaternary carbon peaks at δ 38.7, 38.6, 40.6, 42.31, and 77.31

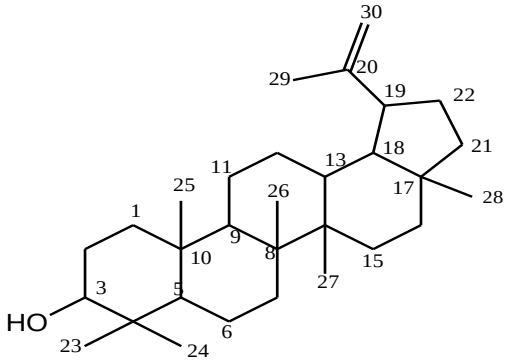
(oxygenated sp^3 quaternary carbon) were also confirmed. Oxygenated sp^3 methine at C-3 was observed at δ_C 78.7. The methyl at C-28 in case of lupeol is oxidized biosynthetically to COOH then decarboxylation removes C-28. Further oxidation of C-17 eventually put hydroxyl group at C-17, and also confirmed from DEPT-135. Oxygenated sp^3 methine at C-3 was observed at C $_\delta$ 78.7.

Table 6 ^{1}H NMR and ^{13}C NMR Spectroscopic data of Lupeol (**17**) [71] and compound (**18**)

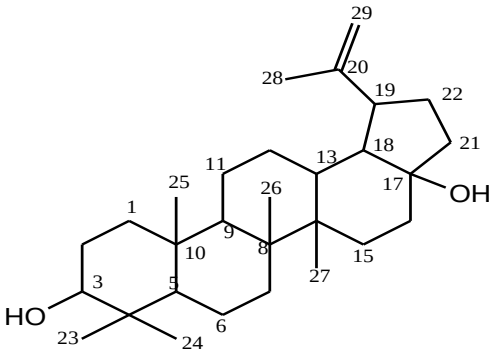
Carbon number	δC of proposeds tructure (**12**)	δH (mult., J in Hz) of compound (**12**)	Carbn number	δC of Lupeol [70]	δH (mult., *J* in Hz) of Lupeol [70]
1	38.7	0.90−1.92 (2H, m)	1	38.7	1.65 (1H, *m*); 0.90 (1H, *m*)
2	26.9	1.60 (2H, m)	2	27.4	1.67 (1H, m); 1.59 (1H, m)
3	78.7	3.20 (1H, dd)	3	79.0	3.20 (1H, *dd*, *J* = 1.5, 5.03)
4	38.7	-	4	38.8	-
5	55.3	0.68 (1H, m)	5	55.3	0.68 (1H, m)
6	18.1	0.90−1.92 (2H, m)	6	18.3	1.50 (1H, m); 1.40 (1H, m)
7	34.2	0.90−1.92 (2H, m)	7	34.3	1.42 (1H, m); 1.32 (1H, m)
8	38.6	-	8	40.8	-
9	50.4	0.90−1.92 (1H, m)	9	50.4	1.29 (1H, m)
10	40.5	-	10	37.1	-
11	20.7	0.90−1.92 (2H, m)	11	20.9	1.40 (1H, m); 1.20 (1H, m)
12	25.4	0.90−1.92 (2H, m)	12	25.1	1.68 (1H, m); 1.07 (1H, m)
13	38.2	0.90−1.92 (1H, m)	13	38.1	1.68 (1H, m)
14	42.3	-	14	42.8	-
15	30.4	0.90−1.92 (2H, m)	15	27.4	1.68 (1H, m); 1.00 (1H, m)
16	32.2	0.90−1.92 (2H, m)	16	35.6	1.48 (1H, m); 1.37 (1H, m)
17	77.3	-	17	42.9	-
18	49.1	0.90−1.92 (1H, m)	18	48.3	1.37 (1H, m)
19	46.9	2.98, ddd (11.0, 11.0, 6.0)	19	47.9	2.98 (1H, ddd) (J=11.0, 11.0, 5.6)
20	150.7	-	20	150.9	-
21	29.5	0.90−1.92 (2H, m)	21	29.8	1.92 (1H, m); 1.37 (1H, m)
22	37.1	0.90−1.92 (2H, m)	22	39.9	1.37 (1H, m); 1.19 (1H, m)
23	27.7	0.85 (3H, s)	23	27.9	0.97 (3H, s)
24	15.2	0.65 (3H, s)	24	15.4	0.76 (3H, s)
25	15.95	0.72 (3H, s)	25	16.1	0.82 (3H, s)
26	15.4	0.88 (3H, s)	26	15.9	0.98 (3H, s)
27	14.6	0.84 (3H, s)	27	14.5	0.94 (3H, s)
-	-	-	28	17.9	0.79 (3H, s)
28	109.3	4.62 (1H, br. s); 4.49 (1H, br. s)	29	109.3	4.7 (1H, br s); 4.61 (1H, br s)
29	19.1	1.50 (3H, s)	30	19.3	1.68 (3H, s)

Note: Carbon number 4, 8, 10, 14, 17, and 20 are quaternary carbons.

Based on the above spectral data and comparison with literature, compound (18) is supposed to be derivative of lupeol (17).



(17)



(18)

Figure 5 Structure of lupeol (17) and proposed structure of compound (18)

4.4 Antibacterial Assays.

The extracts and isolated compounds of *S. guineense* were evaluated for antibacterial activities at a concentration of 200 µg/mL against the four pathogenic bacterial strains one gram positive *Staphylococcus aureus* (ATCC No. 6538) and three gram- negative *Escherichia coli* (ATCC No. 9637), *Klebsiella pneumoniae* (ATCC No. 10031), and *Salmonella typhi*.

Antibacterial potential of crude extracts and isolated pure compounds were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in

Table 7 Zone of bacterial growth inhibition (mm) for crude extract and isolated compounds from the roots of *S. guineense*

	S. aureus	*E. coli*	*S. typhi*	*K. pneumoniae*
DCM: MeOH extract (200 µg/mL)	12	13	13	13
MeOH extract (200 µg/mL)	15	14	17	15
Arjunolic acid (compound 5) (100 µg/mL)	8	6	6	8
Derivative of Lupeol (compound 18) (100 µg/mL)	8	6	6	8
Positive control (Gentamycin) (1mg/mL)	18	16	18	10
Negative control (DMSO)	6	6	6	6

As shown in Table 7, the result revealed that the DCM: MeOH and MeOH extracts of the roots of *S. guineense* showed significant antibacterial activities against the four bacterial strains at 200 µg/mL. Accordingly, the activity of DCM: MeOH and MeOH extract at this concentration against the gram negative [*E. coli* (13 mm, 14 mm), *S. typhi* (13 mm, 17 mm) and *K. pneumoniae* (13 mm, 15 mm) respectively is impressive because Gram negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents.

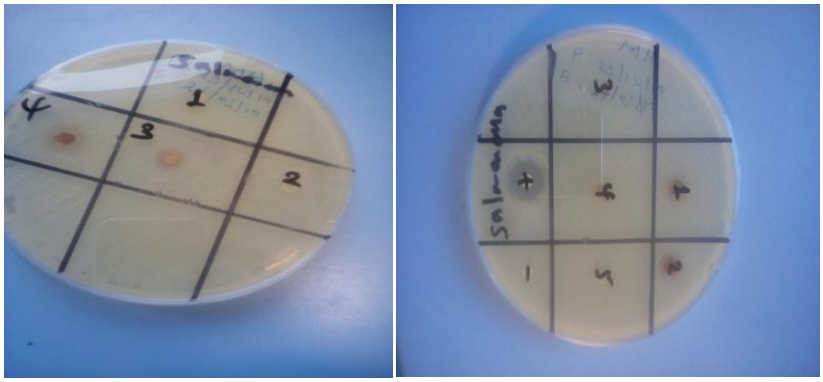
The two isolated pure compounds, arjunolic acid (5) and lupeol derivative, compound (18), showed nearly similar activities against gram positive *S. aureas* with zone of inhibition 8 mm, at 100 µg/mL concentrations. However as the data for antibacterial test assay indicates the compounds have no inhibition effect on the three gram negative bacterial strains.

Although the isolated compounds, arjunolic acid (5) and lupeol derivative, compound (18), showed slight inhibition (8 mm) against *S. aureus*, the crud extracts exhibited the significant activities against the four bacteria (*Staphylococcus aureus, E. coli*, *K. pneumoniae,* and *Salmonella typhi).*

The result of this study showed similarity with the literature report on antibacterial activities of different parts of the plant extracts. It is reported that *S. guineense* extract showed activities against *Staphylococcus aureus, Escherichia coli, Candida albican Salmonella typhi, Klebsiella pneumonia and Pseudomonas aeruginosa* [47]. Tsakala [44] also reported that a dried aqueous decoction of *S. guineense* showed activity against strains of *Salmonella E., Shigella D., Shigella F., E. coli* and *Enterobacter A* ileum.[45] This is the fact which makes the extracts a suitable candidate for investigation in control of antibiotics on these four bacteria. Therefore, plants of the genus Syzygium may prove to be a rich source of compounds with potential anti bacterial activities.

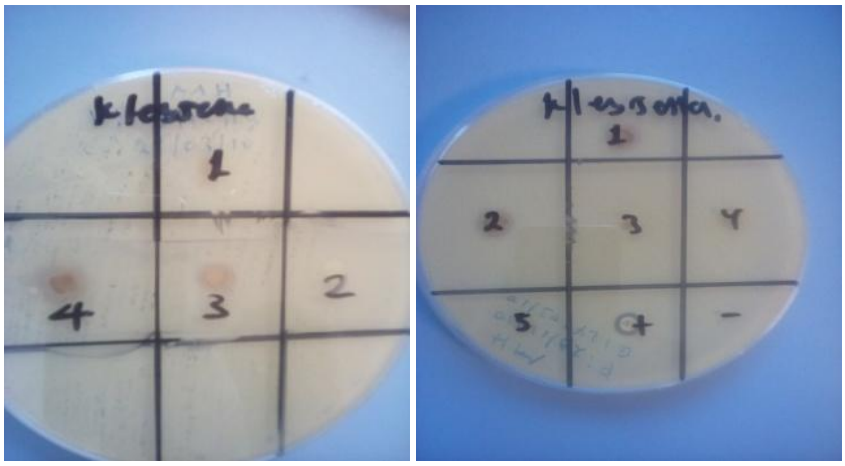
Moreover, the isolated compound, arjunolic acid is reported to have multi functional therapeutic applications in: exerting protection against both type I and type II diabetes, wound healing, preventing palatlate aggregation and in lowering blood pressure [72]. The traditional use of the plant for different ailments such as wound healing, hypertension, etc may be due to the presence of this phytochemical constituent, arjunolic acid. This study confirms the use of medicinal plants is due to the presence of phytochemical constituents.

a) Zone of inhibition bacteria - (*E. coli*)



b) Zone of inhibition bacteria - (*Salmonella typhi*)

c) Zone of inhibition bacteria - (*S. aureus*)



d) Zone of inhibition bacteria - *K. pneumoniae*

Figure 6 Antibacterial activity of the roots of *S. guineense*.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study attempted to isolate phytochemical constituents from the roots of *Syzygium guineense* of Ethiopian flora. Phytochemical screening tests revealed the presence of terpenoids, saponins, flavonoids, tannins alkaloids, phenols and glycosides in both roots extracts. Silica gel column chromatographic separation of the roots extracts of dichloromethane/methanol (1:1) afforded arjunolic acid (**5**) whereas that of methanol extract afforded a steroid lupeol derivative (**18**) isolated for the first time from the roots of the plant. The structures of the compounds were characterized on the basis of spectral data (^{1}H NMR, ^{13}C NMR, DEPT-135 and IR) as well as in comparison with the literature report. In agreement with the previous study, the wide traditional use of the plant may be attributed to its rich steroidal constituents.

The extracts and isolated compounds were evaluated for their antibacterial activities by disc diffusion method against four bacterial strains; one gram positive *staphylococcus aureus* and three gram- negative *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*.

DCM: MeOH and MeOH extracts both showed significant antibacterial activities against the four bacterial strains at concentrations of 200 µg/mL with zone of inhibition 12 mm and above with methanol extract showing highest activity against salmonella typhi (17 mm) were as the two compounds, Arjunolic acid (5) and compound (18), showed moderate activities against *S. aureus* and *K. pneumoniae* with zone of inhibition 8 mm each compared to standard positive control (Gentamycin) (1mg/mL) (18 mm).

5.2 Recommendations

- Comprehensive phytochemical investigation work is recommended so as to isolate polar chemical constituents with the help of advanced instruments such as RP-HPLC
- The results of antibacterial activity of crude extracts and isolated compounds is promising, hence more work is recommended to examine combination of various compounds and extracts with standard drugs and check if there are synergistic effects.
- More comprehensive phytochemical work and biological activity studies should be done on different parts of the plant and examine activity against various strains of microorganisms.

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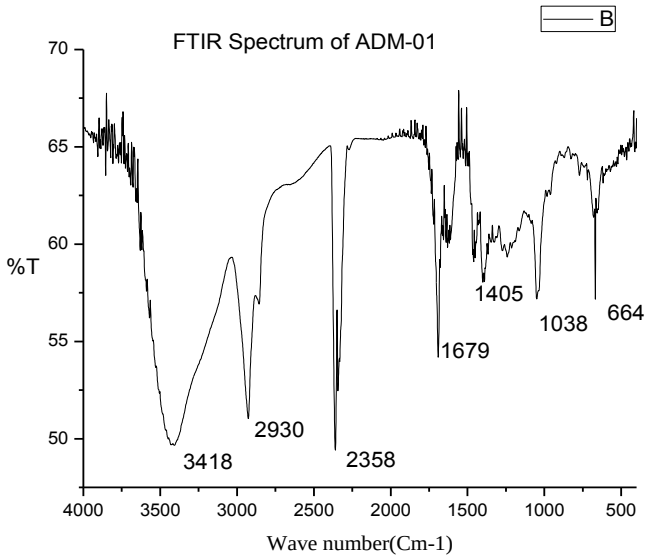
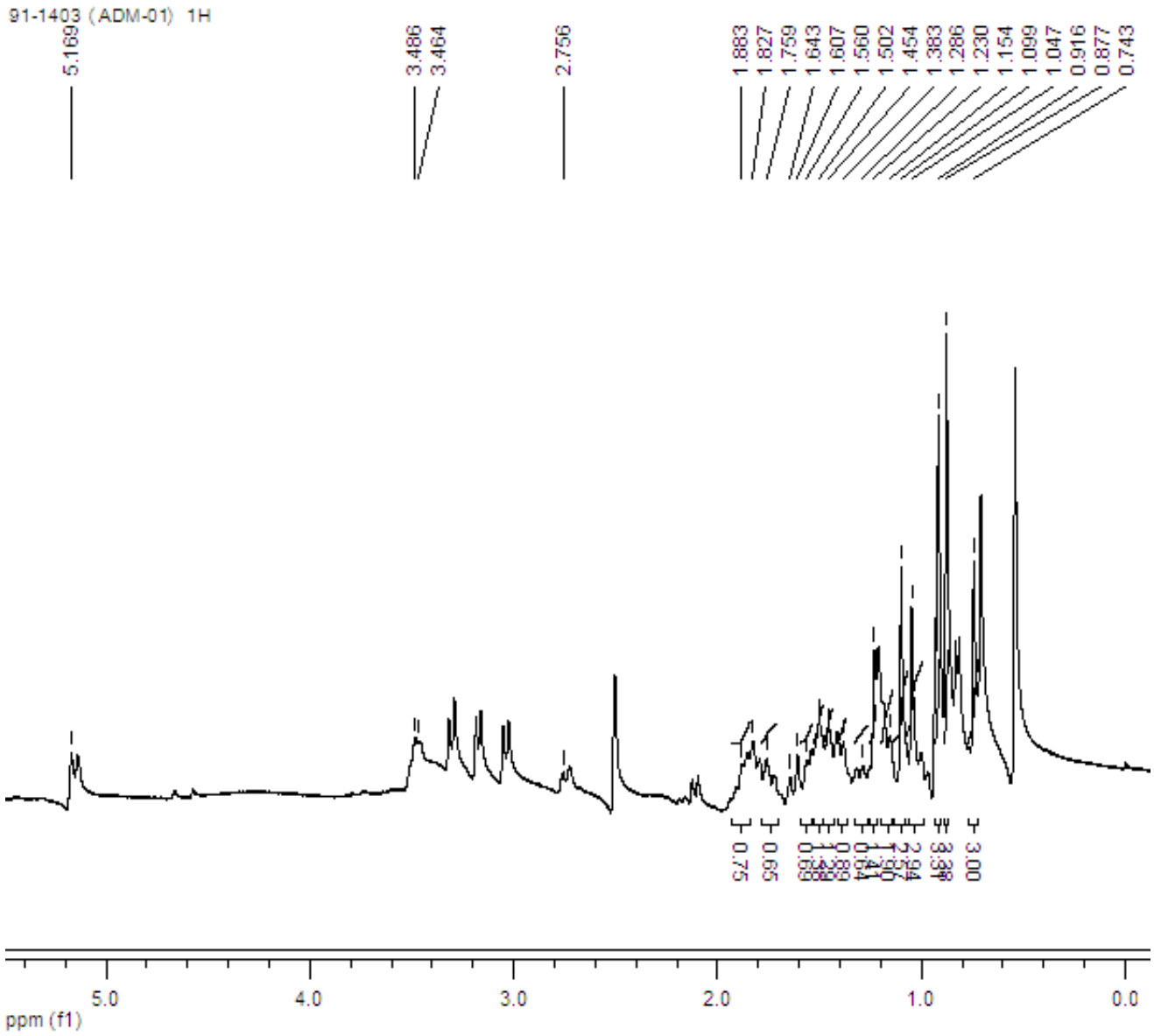
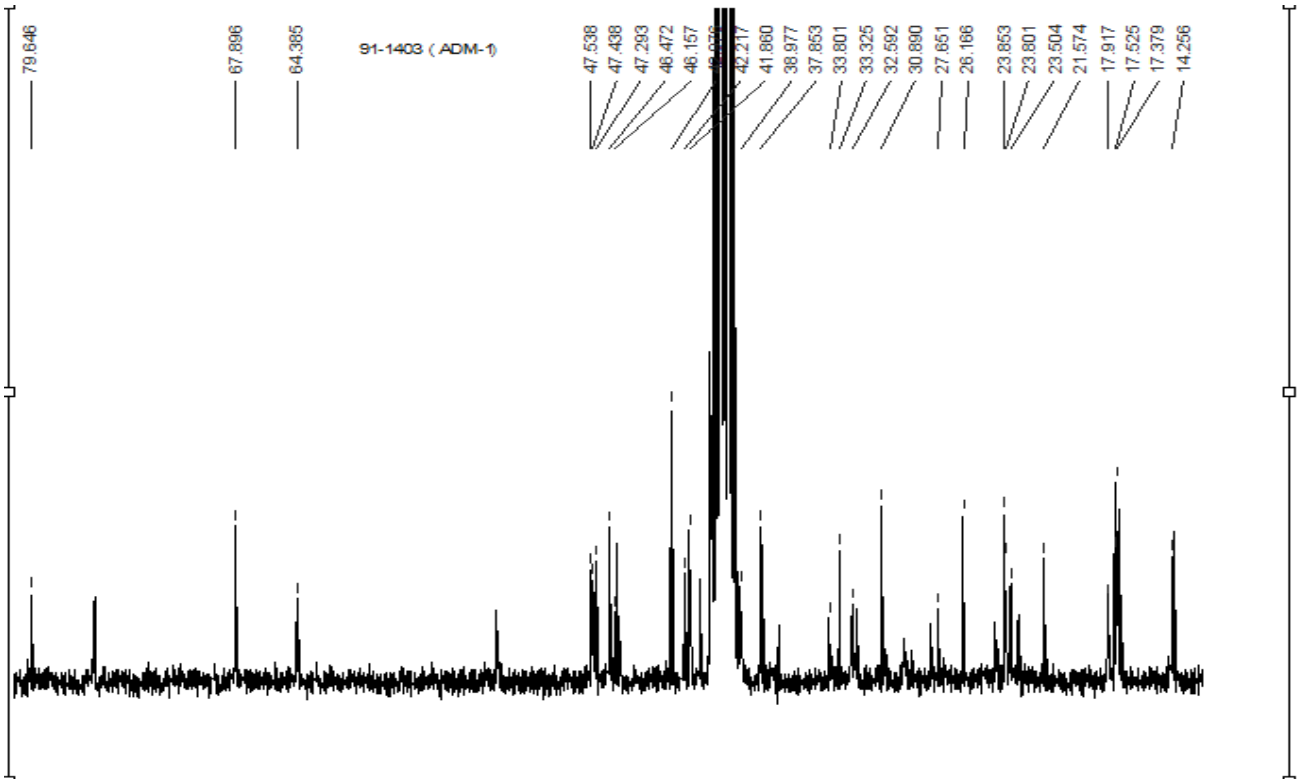
APPENDIX



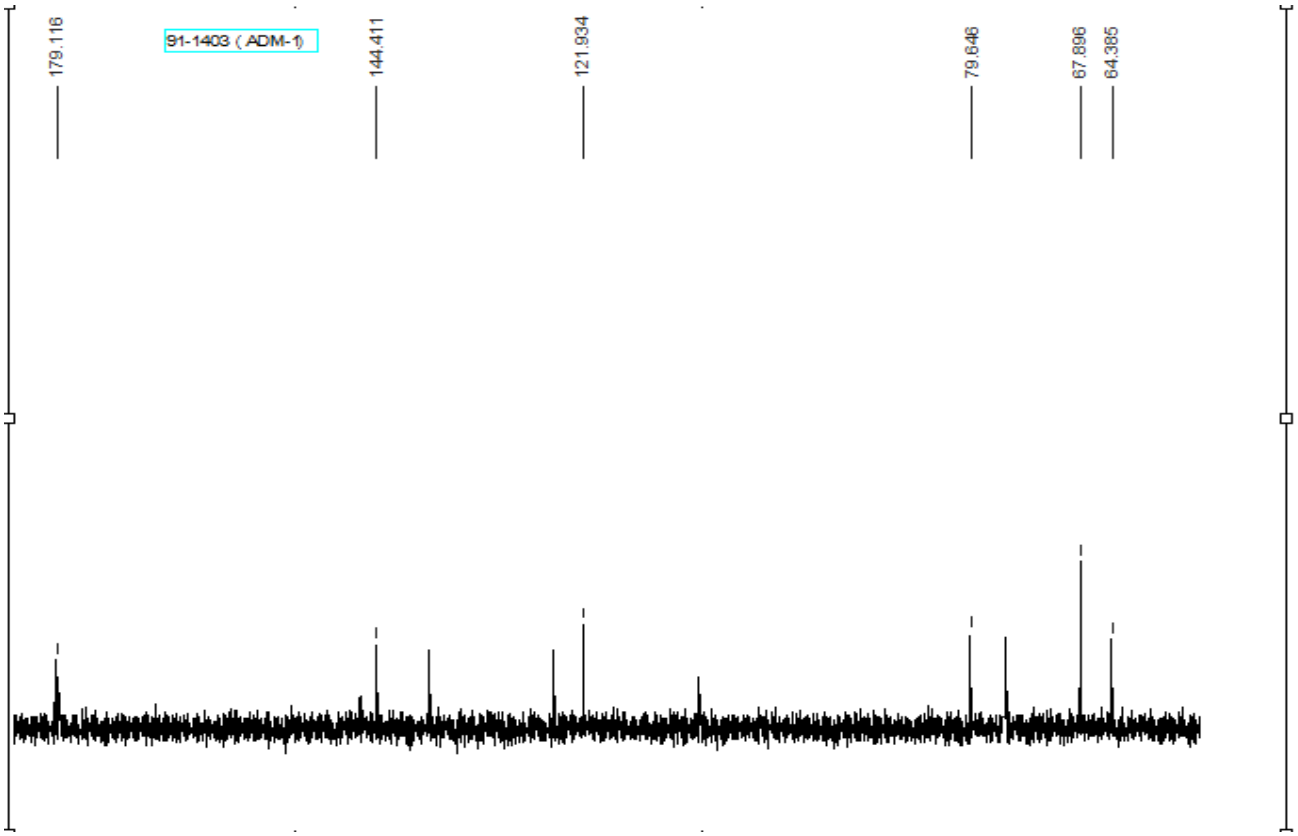
Appendix 1. IR Spectrum of compound (**5**)



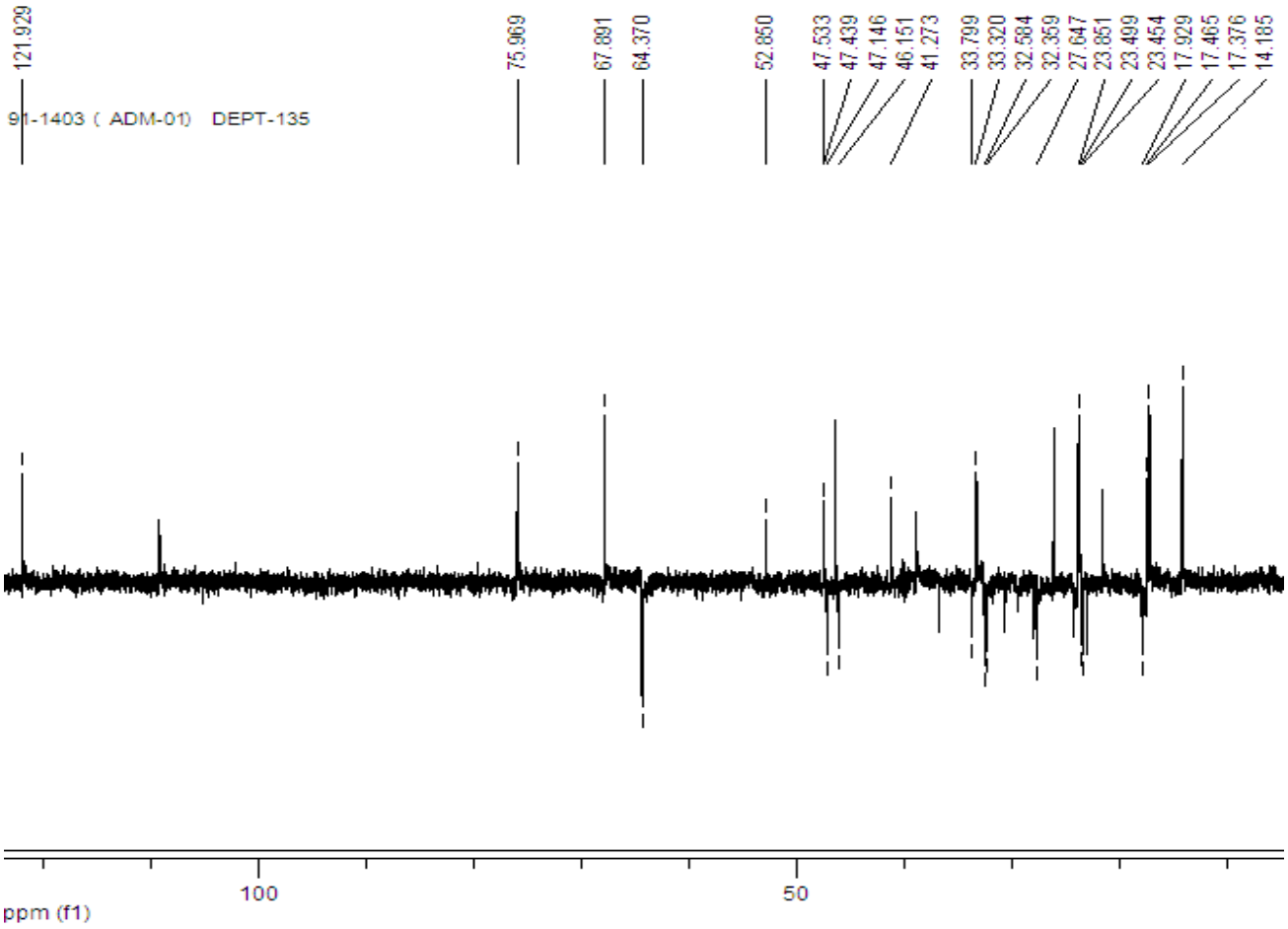
Appendix .**2**. ^{1}H NMR spectrum of compound (**5**)



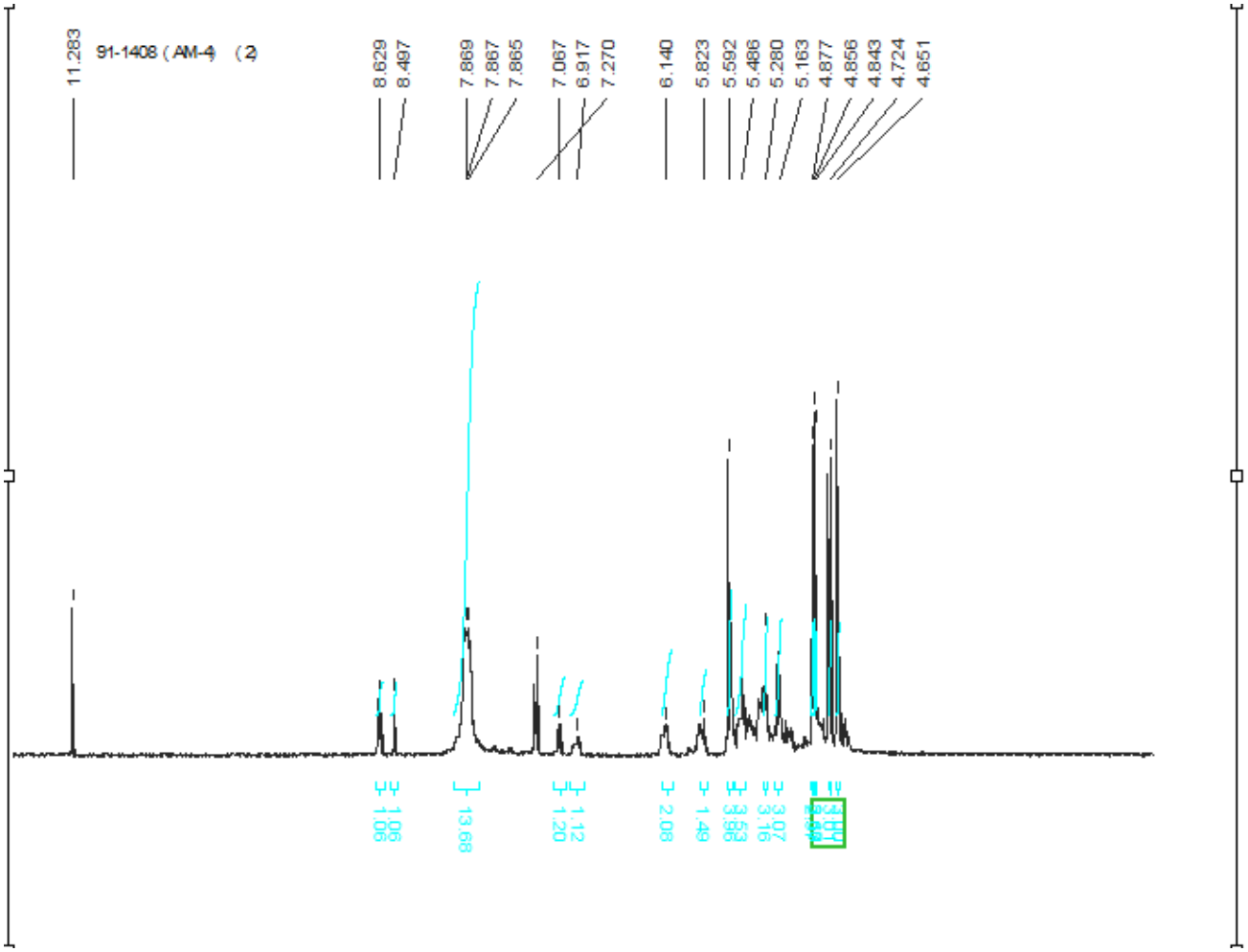
Appendix 3. ^{13}C NMR spectrum of compound (**5**)



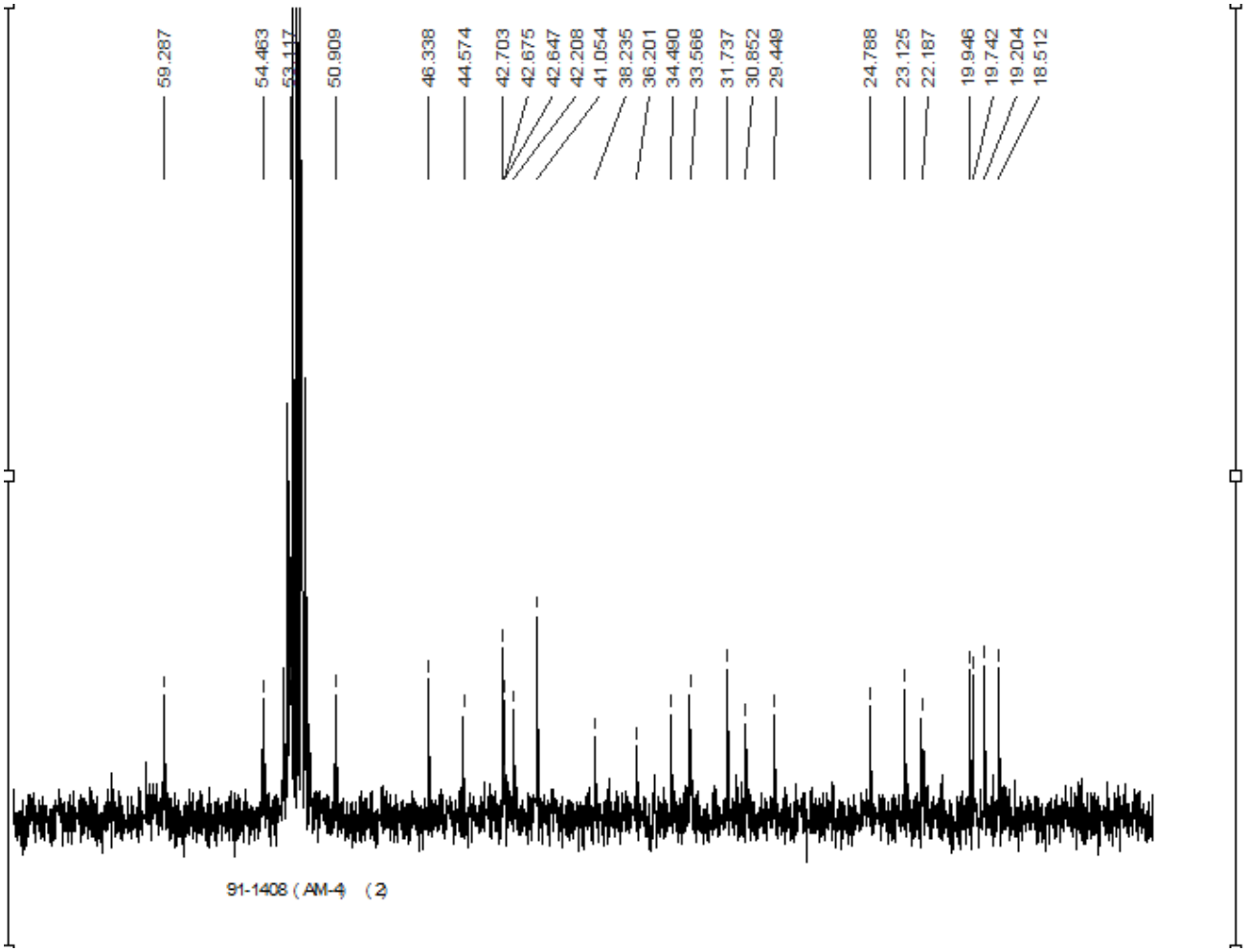
Appendix 4. ^{13}C NMR spectrum of compound (**5**)



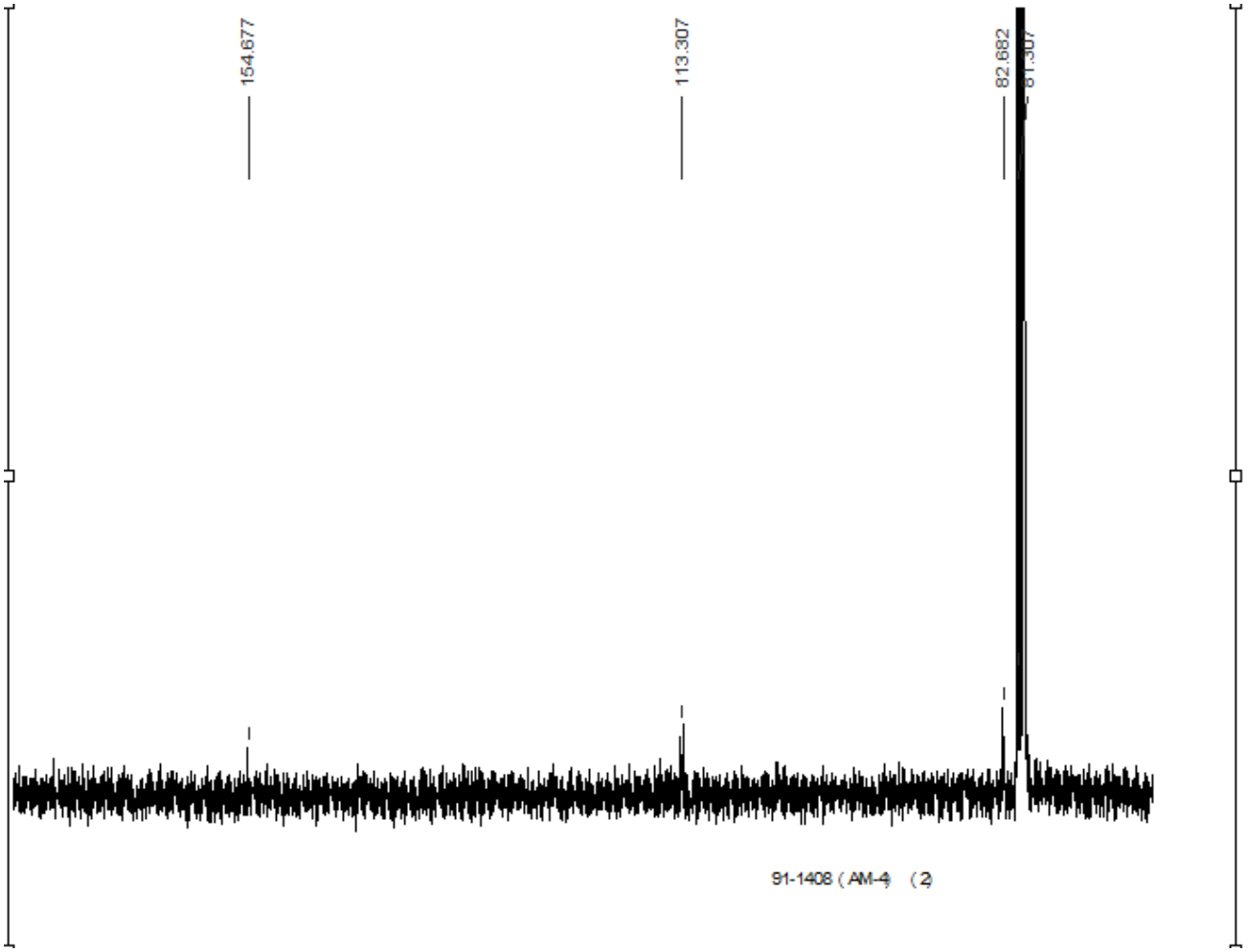
Appendix 4. DEPT-135 spectrum of compound (**5**)



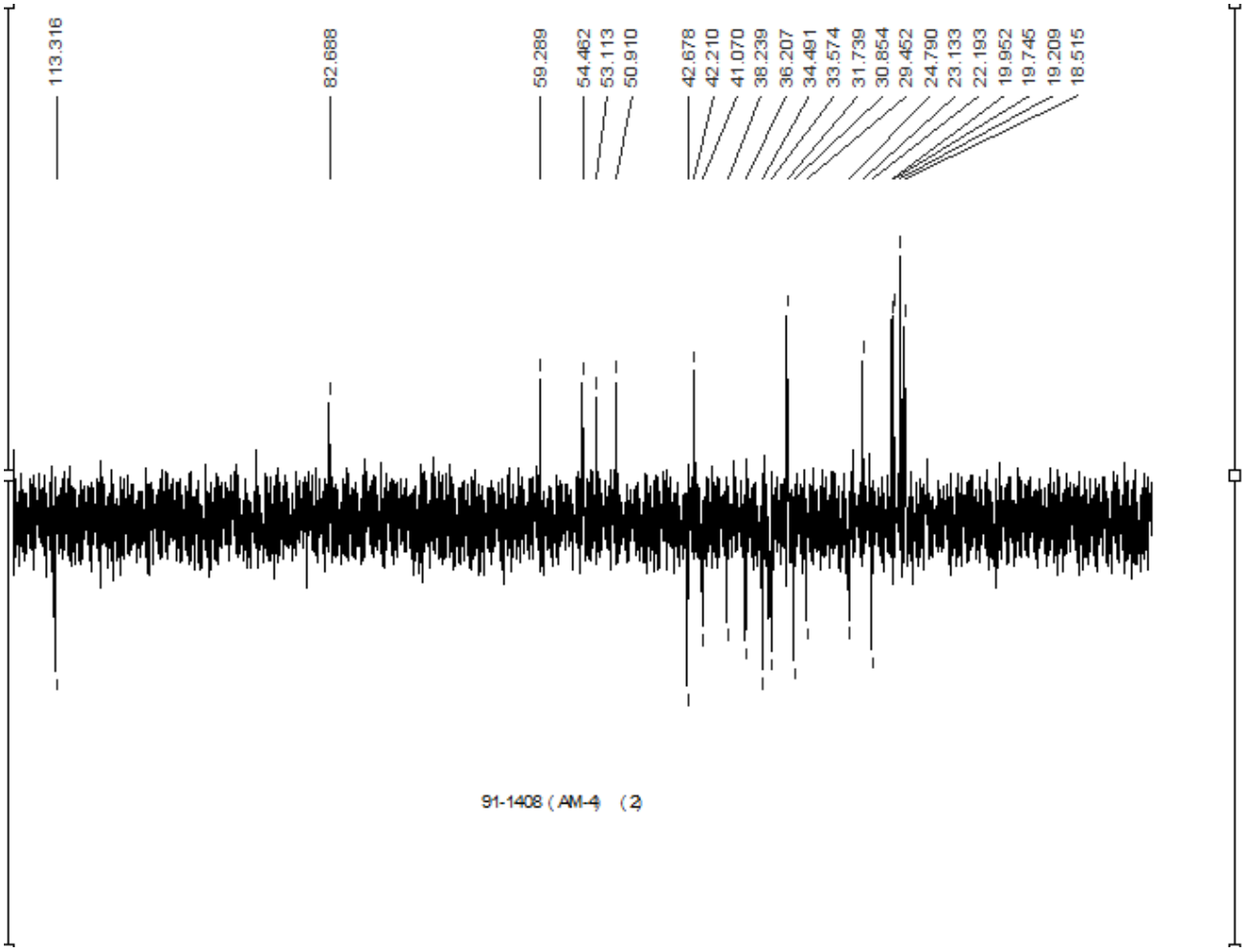
Appendix 5 ^{1}H NMR spectrum of compound (12)



Appendix 6. ^{13}C NMR spectrum of compound (**12**)



Appendix 6. ^{13}C NMR spectrum of compound (**12**) (Cont…)



Appindix7 DEPT-135 spectrum of compound (**12**)