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ON

ISOLATION AND CHARACTERIZATION OF COMPOUNDS FROM THE LEAVES OF *Melia azedarach*AND STEM BARK OF *Albizia schimperiana* AND EVALUATION FOR ANTIMICROBIAL ACTIVITIES

BY

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I, the undersigned confirm that this research work is my original work and has not been presented for any degree or diploma in any other University.

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ACRONYM

B/G/R/S	Benishangul Gumuz Regional State
^{13}C NMR	Carbon Nuclear Magnetic Resonance
DCM	Dichloromethane
DPPH	2, 2-diphenyl-2-picrylhydrazyl
EPHI	Ethiopia Public Health Institute
FTIR	Fourier Transform Infrared
IR	Infra-Red
MIC	Minimum Inhibitory Concentration
^{1}H NMR	Proton Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
TLC	Thin layer chromatography
WHO	World Health Organization

ABSTRACT

Infectious diseases remain a major threat to public health. Despite tremendous progress in human medicine, their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance. Traditional medicinal plants are an important component in the provision of primary health care due to their worldwide availability and fewer side effects. They serve as an alternative to conventional medicines. Thus, the present study was focused on isolation and characterization of compounds from two plants namely; *Melia azedarach* and *Albizia schimperiana*. Accordingly, the leaves of *Melia azedarach*and stem bark of *Albizia schimperiana*were extracted using chloroform/methanol (1:1, v/v) to afford crude extracts. The crude extracts were also subjected to phytochemical analysis for the presence and absence of the common secondary metabolites. In line with, the leaves of *Melia azedarach*was positive for alkaloids, phenols, tannins, saponins, terpenoids and steroidwhereas, the stem bark of *Albizia schimperiana* extract was observed to possess alkaloids, flavonoids, phenols, tannins, saponins, steroid and terpenoids.The chemical study of the leaves extract of *Melia azedarach* andstem bark of *Albizia schimpiriana* afforded two pure compounds whosestructure were established as *β*-sitosterol and α-spinasterol respectively, using standard spectroscopic data (^{1}H NMR, ^{13}C NMR, IR) and literature reports. The crude extracts and isolated compounds were subjected to biological evaluation against four bacterial strains (*Bacillussubtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli)* and two fungus(*Aspergillus flavus* and *Fusariun spp.)*.The two characterized compounds were showed promising antimicrobial activity than the crude extract of both plant species.The observed activity was carried out at concentration of50mg/mL for the crude extracts, 20mg/mL for the isolated compounds, it would be recommended fordetermination of MICvalues provides a quantitative measure for the level of resistance expressed by the test organism.

Keywords: Medicinal plants, *Melia azedarach, Albizia schimperiana,* Phytochemicals, Antimicrobial activity, Disc diffusion.

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Traditional medicine is the oldest form of health care in the world and has been used in the prevention and treatment of various kinds of illnesses. Historically, different societies have developed various useful healing methods to combat health and life threatening diseases [1, 2] which comprise unscientific knowledge systems that developed over generations before the era of western medicine [3]. The knowledge and practice is usually passed on oral base from generation to generation and is carefully protected in certain families [4].

Medicinal plants are the backbone of traditional medicine, in which more than 3.3 billion people in the less developed countries utilize on a regular basis [5]. Moreover, WHO estimated that more than 80% of the world's population still relies on traditional medicine for their primary healthcare needs [6]. It is also important to note that WHO underlined the importance of traditional medicine in the health system, and created strategies, guidelines and standards for botanical medicines [7]. Medicinal plants are not only limited to traditional usage but also considered as a rich source of ingredients in the development of the modern drugs [8]. For example, the discovery of modern drugs such as quinine, vincristine, digoxin and digitoxin, artemisinin, etc from medicinal plants signifies the huge potential that still exists for the production of many more novel pharmaceuticals [9].

WHO estimates that nearly 20,000 medicinal plants do exist and wide spread over in 91 countries including 12 mega biodiversity countries (the world’s top biodiversity-rich countries including Australia, Brazil, China, Colombia, Democratic Republic of Congo, Ecuador, India, Indonesia [10]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, scientists turned to ethno-pharmacological and found literally thousands of phytochemicals from plants and other nature-based

sources as safe, and broadly effective alternatives with less adverse effect. These products were reported to possess a wide range of beneficial biological activity such as anticancer, antimicrobial, anti-malaria, antioxidant, antidiarrheal, analgesic and wound healing [10].

1.2 Active components of plant extracts

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in plants [11]. They are classified in to three large molecular families according to their biosynthetic pathways: phenolics, terpenes and steroids, and alkaloids. Phenolic compounds are known for their potency as antioxidants and free radical scavengers, acting as hydrogen donors, reducing agents and singlet oxygen quenchers [12]. Flavonoids, which are a subclass of phenolics, are known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [13]. Terpenoids are condensation products of C5 isoprene units which are important constituents of essential oils [14]. They have been shown to be active against bacteria, fungi, viruses, and protozoa. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds [13]. Alkaloids are the best known nitrogen-containing metabolites of plants and are sparsely distributed in the plant kingdom, but much more specific to defined plant genera and species. This is probably due to the limited supply of nitrogen in plants [15].

1.3 Antimicrobials

Antimicrobials are substances that kill or inhibit the growth of microorganisms in the form of antibiotics, which are products of microorganisms or synthesized derivatives [13]. Different types of antimicrobials exist: antibiotics, anti-viral, anti-fungal, anti-protozoan etc. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources [16]. Most anti-viral, anti-fungal, anti-protozoa and anti-cancer drugs, however are obtained from synthetic sources. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in

immune compromised patients in developed countries [17]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with side effects. There is an urgent need to search new infection-fighting strategies to control microbial infections [18]; however the development of new antibiotics should be continued as they are primary importance to maintain the effectiveness of antimicrobial treatment [19]. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin [20].

Despite the extensive use of antibiotics and vaccination program, infectious diseases continue to be a leading cause of morbidity and mortality worldwide because of their resistance to antibiotics [21]. In order to find novel antimicrobial agents with new modes of action, plants have been explored as sources for the identification of new and effective antimicrobials [22]. This further justifies the search for alternative products from plants used in folklore medicine. Many plants growing in the studying area are cleaned for agricultural purposes. This has resulted in drastic reduction of very useful medicinal plants by lack of documented data to support the usefulness of these plants for conservation. The aim of this study was to identify chemical constituents found in the leave extract of *Melia azedarach* and stem bark of *Albizia schimperiana* that would be a potential against bacterial and fungal pathogens.

1.4 Statement of the problem

According to WHO infectious diseases are the number one cause of deaths worldwide, which account for more than 50 % of the deaths in tropical countries. To combat these diseases in the last few decades, pharmaceutical industries have produced a number of antibiotics, but the resistance of microbes has also increased parallelly to almost all the antibiotics that are available in the market. This has resulted multiple drug resistance in both human and plant pathogens due to indiscriminate use of synthetic drugs especially in the developing countries [23].This resistance have been attributed to overdose and under dose of drugs, due to over counter prescription of drugs, ability of microorganisms to undergo genetic variability (mutation). Furthermore, some antibiotics have serious undesirable side effects which limit their applications. Moreover, the toxicity and side

effects of the conventional drugs have also become increasingly unmanageable, while their costs are expensive. There is an urgent need to search new antimicrobial drugs with new modes of action, which could be safer and effective from natural sourcesto combat these infection diseases. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin.

1.5 Research questions

I. Do these medicinal plants possess common secondary metabolites in their crud extract?
II. Do the leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana* active against growthof pathogenic organism?

1.6 Objective of the study

1.6.1 General objective

i. The general objective of the study is to identify secondary metabolites from the leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana* for antimicrobial principles.

1.6.2 Specific objectives

i. To identify secondary metabolites from the leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana*
ii. To isolate and characterize secondary metabolites from leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana* using chromatographic techniques;
iii. To elucidate the structures of the isolated compounds using spectroscopic methods such as NMR, IR;
iv. To evaluate the antimicrobial activities of the crude extracts and isolated compounds from both plant species against four bacterial strains

(*Bacillussubtilis*and *Staphylococcus aureus)* and two fungi species i.e. *Aspergillus flavus* and *Fusarium spp.* using disc diffusion methods.

1.7 Significance of the study

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their world-wide availability and fewer side effects. Therefore, scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. Traditionally, different parts of *Melia azedarach* has been used by the local people for the treatment of diarrhea, malaria and various types of skin diseases; particularly, the leaves of this plant is used for the treatment of hemorrhoid, pest control, and even used around bed room instead of net to protect mosquito and other insects' bite. *Albizia schimperiana* isalso locally used for the treatment of various bacterial infections (cough and diarrhea), antihelmintic activities, antimalarial activities, skin diseases. Therefore, the present work is to verify the efficacy of these plants and the antimicrobial activity of the crude extracts and the isolated compounds against selected micro-organisms. The obtained result from the study would be helpful in supporting and promoting the usage of these plants as antimicrobial and their conservation by the communities. Moreover, the bioactivity results of both medicinal plantsprovided preliminary scientific justification for the traditional medicinal uses and an important step towards its acceptance and development of modern drugs.

CHAPTER TWO

2. LITERATURE REVIEW

2.1.1 Geographic distribution of *Melia azedarach*

Melia azedarach(locally known as "Mimi")is one of the most useful traditional medicinal plants, which its name was derived from the classical Greek word "*Melia*" for the manna ash or flowering ash, referring to the similarity of the leaves to that plant and *azedarach*from the name of an ancient poisonous tree [24, 25].*Melia azedarach* is native to tropical Asia but also widely distributed in Pakistan, India, Indonesia, Southeast Asia and Australia. It has become naturalized in different countries including Philippines, United States of America, Brazil, Argentina and many African and Arab countries [26].

2.1.2 Botanical information of *Melia azedarach*

The family Meliaceae to which *Melia azedarach* belongs contains 45 genera and over 750 species [27]. *Melia azedarach* is a small to medium sized deciduous tree growing to a height of 5 to 15 m tall, and 30 cm to 60 cm in diameter. This plant is usually characterized by the presence of a spreading, dense and dark green crown. Its bark is dark brown in color, relatively smooth, and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green and relatively pale. Flowers are white with purple stripes and are characterized by the presence of a typical fragrance. Fruits or berries are yellow, round, smooth, and fleshy. Dried fruits are hard with seeds [28].

(A) Leaves(B) Fruits(C) whole parts

Figure 1:Picture of *Melia azedarach* leaves, fruits and its whole parts takenfrom Tongo Town by FD in August, 2017

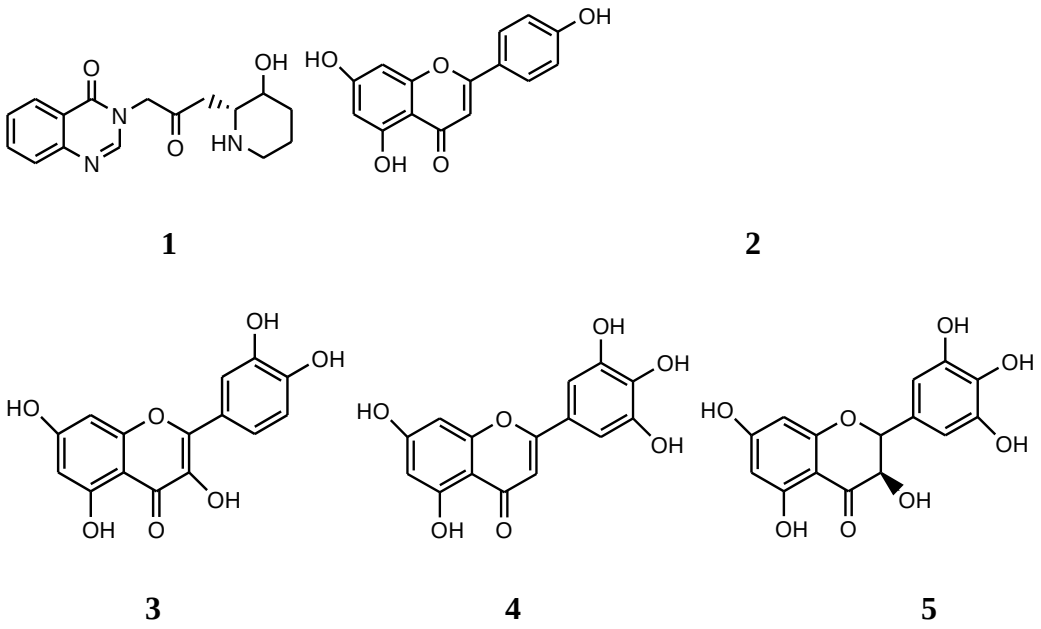
2.1.3 Traditional medicinal uses of *Melia azedarach*

Medicines that are used today are not definitely the same as those that were used inancient times or even in the recent past. Severalmodifications, improvement, sophistication and newer discoveries contribute continuously to the type, quality, presentation and concept of medicinal preparation. *Melia azedarach* possesses anumber of medicinal properties; various preparations of this plants are being used for the treatment of several diseases [29]. There are several reports on the analgesic, anticancer, antiviral, anti-malarial, antibacterial, antifungal, stomach ache, intestinal disorders, uterine illnesses and anti-fertility activity of this plant [30]. The powder of the dried fruits of this plant is claimed to be an effective therapy for the treatment of diabetes [31].

2.1.4 Compounds isolated from the familyMeliaceae

Meliaceae is distributed in tropical and subtropical regions. Many plants of Meliaceae family possess insecticidal properties against fecundity and fertility of mosquito vector and are at the same time very eco-friendly [32]. Various classes of chemical constituents were isolated from different parts of Meliaceae members. Chemically, the Meliaceae is characterized by synthesis of modified limonoids. Over 300 limonoids have been isolated today and they are more diverse and abundant in this particular family than in any other

family. Several triterpenoidal derivatives were also isolated from different genera of Meliaceae. Among different members of Meliaceae, *Azadirachta indica* had been extensively studied for its bioactive chemicals [33]. Some of the isolated compounds from various parts of the family Meliaceae, include febrifugin **(1)** from the bark [34] and flavonoidsnaringenin **(2)**, quercetin **(3)**, myricetin **(4)** and dihydromyricetin **(5)** from the wood [35].



1 **2**

3 **4** **5**

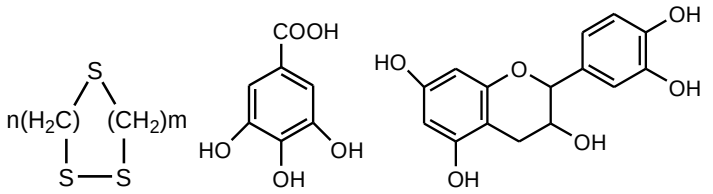
Figure 2: Some of the chemical structures of the isolated compounds from the familyMeliaceae

2.1.5 Biological activities of the genus *Melia* and its isolated compounds

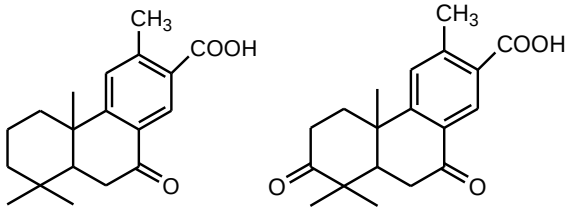
Various classes of chemical constituents were isolated from different parts of the genus *Melia* as in (Table 1). In recent past, the genus *Melia* has been characterized by the production of limonoids. It was also observed that some species of this plant has many similarities in chemical profile as well, thus synonymous to each other [36]. The plant is well known as a rich source of biological activities including insecticidal, antifeedant, growth inhibitor, antimicrobial, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans [37].Some of previously isolated and reported chemical constituents from the genus *Melia* showed in (Table 1).

Table 1:Some of the compoundsisolated from the genus *Melia*

Name of the isolated compounds	Plant parts	References
Cyclic trisulphide **(6)**	Leaf	[38]
Gallic acid **(7)**, (–) epicatechin **(8)**	Bark	[39]
Margolone **(9)**, margolonone **(10)**	Bark	[40]
Azadirachtin**(11)**	Nodal callus	[41]
Azadironolide **(12)**	Fruit coats	[42]
12-hydroxy amoora statin **(13)**	Stem	[43]
Tetranortriterpenoids **(14)**	Roots	[44]
Limonoids spirosendan **(15)**	Root bark	[45]
12 -hydroxykulactone**(16)**	Seeds	[46]
2α,3β - dihydro-5-pregnen-16-one **(17)**	Leaves	[47]



6 **7** **8**



9 **10**

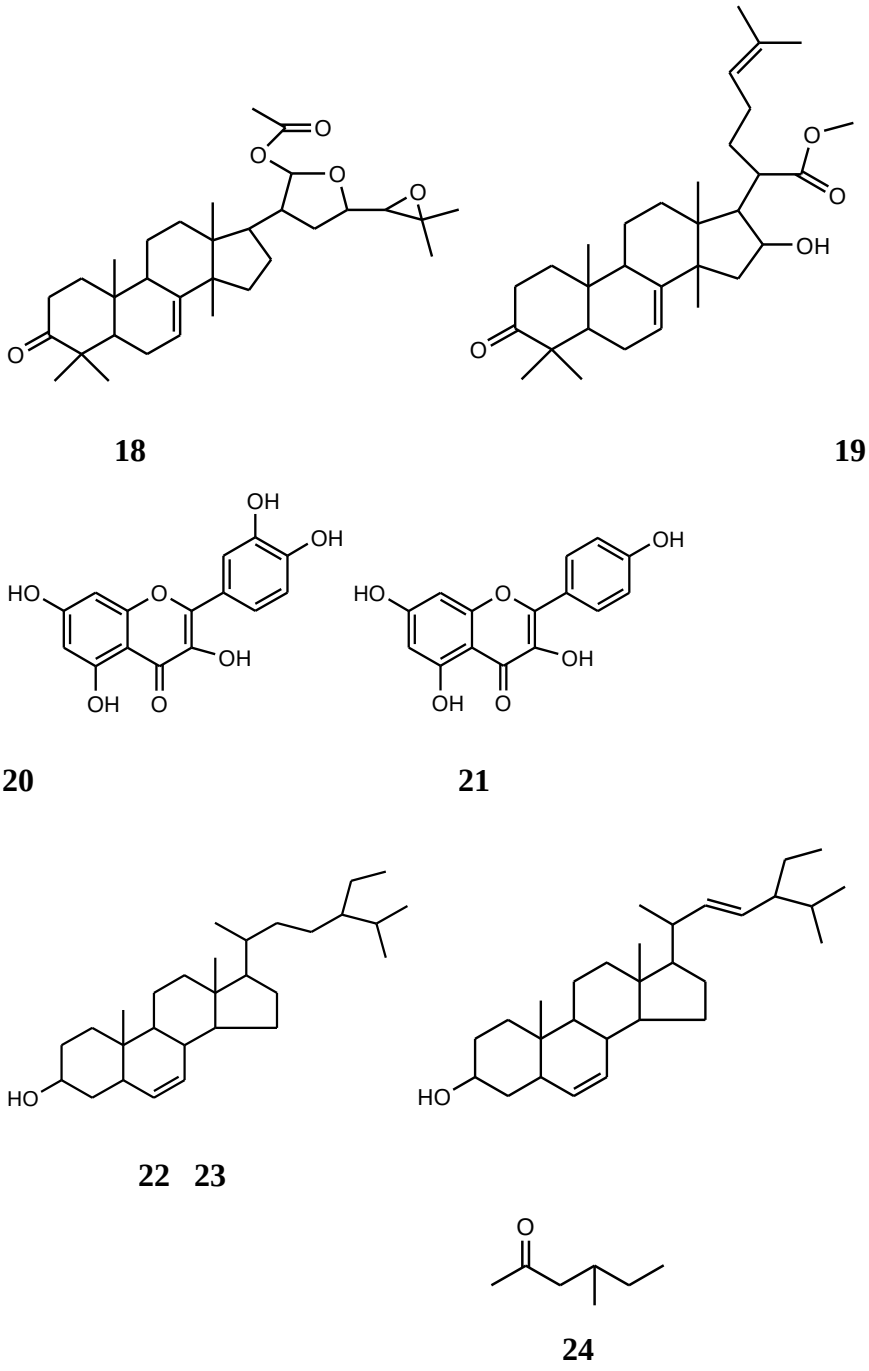
Figure 3: Some of the chemical structures of the compounds isolated from the genus *Melia*

2.1.6 Phytochemistry of *Melia azedarach*

Melia azedarach contains a number of organic molecules *i.e.* flavonoids, terpenoids, steroids, acids and anthraquinones [48]. Some of these compounds have been reported to have biological properties such as anti-fertility, antimalarial, antifungal, antibacterial, wound healing, antioxidant and antiviral activities. Triterpenoids constitute a wide biologically interesting group of terpenoids and include a large structural diversity of secondary metabolites with more than 100 carbon skeletons identified from terrestrial and marine living organisms. This class of natural products including triterpenes, steroids, limonoids, quassinoids and triterpenoidal and steroidal saponins, consists of over 30,000 compounds isolated and identified [49]. Steroids possess a fully or partially reduced cyclopenta-phenanthrene scaffold, sometimes bearing methyl groups at C-10 and C-13. However, the backbone of the side chain at C-17, its length and the stereochemistry of some of its chiral centers lead to different steroid skeletons [50]. Some of previously isolated and reported chemical structure from different parts of *Melia azedarach* showed in (Table 2).

Table 2: Some of the compoundsisolated from different parts of *Melia azedarach*

Name of the isolated compounds	Name of the plants (Parts)	References
β-acetoxymelianone **(18)**	Fruit	[51]
methyl kulonate **(19)**	Fruit	[52]
Kampherol**(20)**	Leave	[53]
Quercetin**(21)**	Leave	[53]
β-sitosterol **(22)**	Root	[54]
Stigmasterol **(23)**	Seed	[54]
4-methayl-2-hexanone **(24)**	Leave	[55]



18 **19**

20 **21**

22 **23**

24

Figure 4: Some of the chemical structure of the compoundsisolated from different parts of *Melia azedarach*

2.1.7 Biological activities of the *Melia azedarach*

2.1.7.1 Antifertility activity of *Melia azedarach*

It was reported that among several methods of contraception, some of them have serious adverse effects, such as hormonal imbalance, hypertension, and increased risk of cancer and weight gain, therefore, search for new antifertility molecule with minimum side effects continues which eventually led to the hydro-alcoholic extract of *Melia azedarach* roots for anti-implantation, anti-estrogenic and anti-progestational activities. The result obtained from this work showed that the extract exhibited significant anti-implantation and anti-progestational activity. This finding appears to indicate that certain chemical constituents are present in the extract which impairs the synthesis, secretion and functions of ovarian steroids by blocking the implantation process [56].

2.1.7.2 Antioxidant activity of *Melia azedarach*

Antioxidants are natural occurring plant substances that protect the body from damage caused by harmful molecules called free radicals. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. The high DPPH scavenging activity of *Melia azedarach* was observed which may be due to hydroxyl groups present in the phenolic compounds of this plant. The antioxidant effect of phenolic compounds is by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals. The $^{\cdot}OH$ groups in phenolic compounds are considered to have a significant role in antioxidantactivity. It is reported, the antioxidant activity of phenolic compounds is be mainly due to their redox properties [57].

2.1.7.3 Antiviral activity of *Melia azedarach*

A peptide “meliacine” isolated from*Melia azedarach*leaves was found to inhibit the multiplication of foot and mouth disease viruses [58]. It was also reported that another compound “meliacarpin” found in the purified extract of *Melia azedarach* leaves inhibits the vasicular stomatitis and Herpes simplex virus multiplication in vitro when added after infection with no cytotoxic effects [59].

2.1.7.4 Antibacterial activity of *Melia azedarach*

A study was conducted on antibacterial potential of the polar and non-polar extracts of the seeds of *Melia azedarach* against eighteen hospital isolated human pathogenic bacterial strains. Petrol, benzene, ethyl acetate, methanol, and aqueous extracts at five different concentrations (1, 2, 5, 10 and 15 mg/ml) were evaluated using disk diffusion method. All extracts of the seeds showed significant antibacterial activity against tested *Basillus subtilis*, *Shigella flexeneri*, *Plesiomonas shigellides*, and *Staphylococcus aureus*. However, ethyl acetate extract revealed the highest inhibition comparatively among all other extracts. Therefore, this study also favored the traditional uses of *Melia azedarach* reported earlier [60].

2.1.7.5 Antimalarial activity of *Melia azedarach*

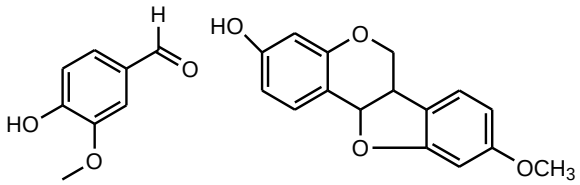
Antimalarial effect of methanol extract of fruit, bark and leaves of *Melia azedarach* was studied on mice against the malaria parasite *Plasmodium berghei*. The study showed that fruit and bark extracts have significant suppression effect on parasitaemia. It concluded that *Melia azedarach* has significant anti-malarial effect but less significant than chloroquine [61].

2.1.7.6 Wound healing activity of*Melia azedarach*

Wound healing potential of methanol leaf extract of *Melia azedarach* possesses significant wound healing activity in alloxan induced diabetic rats. Delay in wound healing process in diabetes mellitus believed to be largely caused by some basic mechanisms, such as increased blood sugar that impairs blood flow and the release of oxygen, impaired local immune and cell defenses and microbial infections. *Melia azedarach* leaf extract enhanced the wound healing in diabetic rats which is believed to be due to its antimicrobial activity [62].

2.1.7.7 Fungicidal potential of *Melia azedarach*

The activity of ethanolic leaf, seed and fruit extracts from *Melia azedarach* in controlling plant and human pathogenic fungi such as *Aspergillus flavus, Fusarium monitiform, Microsporum canis* and *Candida albicans*has been reported [63].Three compounds were isolated from crude extracts and identified as, vanillin **(25)**, hydroxyl-3-methoxcinnamaldehyde **(26)** and (±) pinoresinol**(27)**. In a subsequent research effort, the seeds of ripe fruits from *Melia azedarach* were utilized to isolate the active compound scopoletin **(28)**, a hydroxyl coumaramin, and the subsequent testing of its antifungal synergestic effect. Results revealed a good antifungal activity of the isolated compounds when tested against *Fusariumverticilloides* as well as its synergistic effect when it was combined with two conventional fungicides mancozeb or carboxin.



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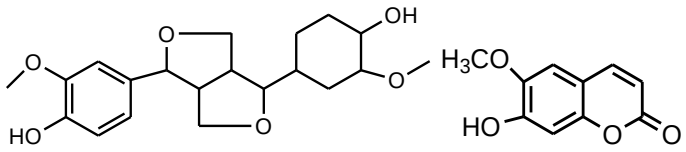


27 **28**

Figure 5: Some of fungicidal potential compounds from different parts of *Melia azedarach*

2.2.1 Geographic distribution of *Albizia schimperiana*

Albizia schimperiana (locally known as ‘Hambabeesa’ in Afaan Oromo and 'Sesa’ in Amharic) is an evergreen tree that grows at an altitude of 1600-2600 m [64]. Most of these plants are fast-growing subtropical and tropical trees and shrubs. The genus *Albizia* comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa. Leaves are bipinnate with leaflets in numerous pairs or larger in fewer pairs. Flowers are in globose heads or spikes. Stamens elongate and are usually white. Fruit is broadly linear indehiscent or 2- valved, valves not twisted [65].

(A) whole parts

(B)Leaves (C)Stem bark

Figure 6:Picture of *Albizia schimperiana* leaves, Stem bark and its whole parts taken from Ginigo Guduru kebele by FD in August, 2017

2.2.2 Botanical information of *Albizia schimperiana*

Among the vast diversity of plants, there are three subfamilies of the legume family which are Papilionoideae, Caesalpinioideae and Mimosoideae. Members of the subfamily Mimosoideae have flowers with radial symmetry. This subfamily includes the genera, *Acacia, Albizia, Samanea,Prosopis* and *Calliandra. Albizia schimperiana*belongs to the family Fabaceae which consists of 650 genera and about 18,000 species distributed in most part of the world. The genus *Albizia* includes some 100-150 species throughout the tropics [66]

2.2.3 Traditional medicinal values of *Albizia schimperiana*

Albizia schimperiana is used as a traditional medicine for the treatment of various infections such as bacterial infections, parasitic infections, and stomach pain [67]. The leaf of this particular plant has shown significant antimicrobial activity on different bacterial species and potential antihelmintic activity [68]. The roots are used to treat headache [69], cure skin diseases and other pains [70] and also used to treat tuberculosis, infertility of women and as an aphrodisiac [71]. The stem bark is used to treat cancer related diseases as warts [72] and the bark is used to treat malaria [73].

2.2.4 Traditional medicinal values of the genus *Albizia*

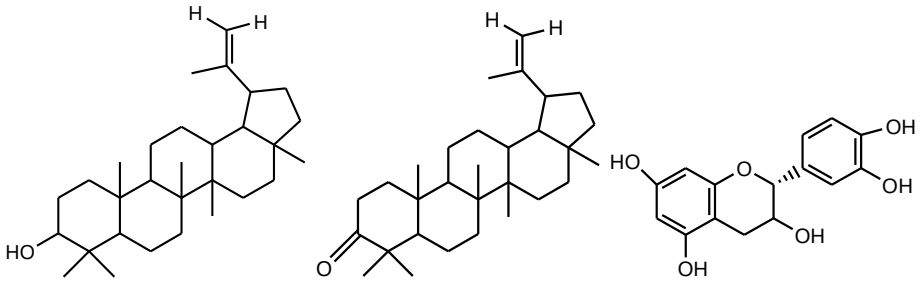
The current literature revealed that some plants belonging to genus *Albizia* have great medicinal values [74]. The flowers are being commonly used to treat anxiety, depression and insomnia in traditional Chinese medicine. *Albizia* species are socially significant for producing high quality timber and as a valuable resource for gum yield. Many extracts of different species of genus *Albizia* have been reported to have many pharmacological activities, such asantimicrobial activity of *Albizia ferrugenia* [75] and *Albizialebbeck* [67] antidiabeticactivity of *Albiziaodoratissima* [77], and anti-depressant activity of*Albizia julibrissin* [78]. Species like *Albizialebbeck* is used by some cultures to treat boils, cough, lung problems, abdominal tumors and also shown high potential in soil redevelopment process during early phase of mine spoil restoration in dry tropical environment.

2.2.5 Compounds isolated from the genus *Albizia*

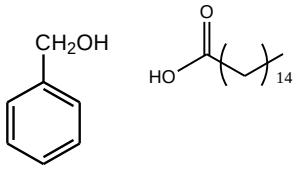
Phytochemical investigation of different *Albizia* species revealed the presence of different classes of secondary metabolites, such as saponins, terpenes, alkaloids and flavonoids, but most of the phytochemical studies done on different *Albizia* species lead to the isolation of saponins. Saponins have been used extensively in drug-related industry due to their pharmaceutical properties; which has driven the emergence of new extraction technologies with the main purpose of optimizing the yield in order to accommodate their need [79]. The crude extract of *Albizia Schimperiana* confirms the presence ofsecondary metabolites such as phenolic compounds, tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones [80]. Some of previously isolated and reported chemical constituents from the genus *Albizia*showed in (Table 3).

Table 3: Some of the isolated compounds from the genus*Albizia*

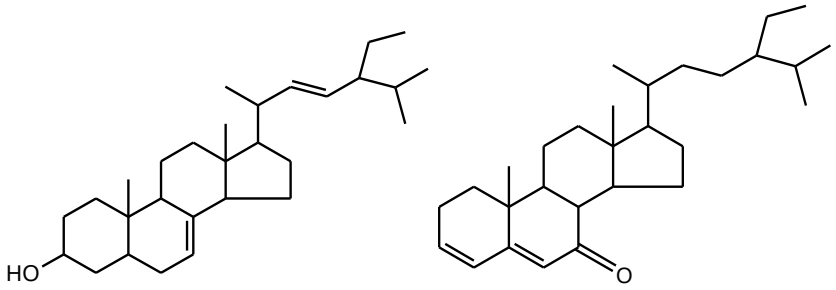
Name of the isolated compounds	**Investigated Part(s)**	**References**
Lupeol **(29)**	stem bark	[81]
Lupenone **(30)**	Leaves	[81]
Catechin **(31)**	stem bark	[82]
Benzyl alcohol **(32)**	stem bark	[83]
n-hexadecanoic acid**(33)**	Heartwood	[84]
Chondrillasterol **(34)**	Heartwood	[85]
Stigmasta-3,5-dien-7-one **(35)**	steam bark	[86]
Friedelan-3-one **(36)**	stem bark	[87]
Pyrogallol**(37)**	Root	[88]
Resorcinol**(38)**	Root	[88]
Adianthifoliosides **(39)**	Root	[89]
Albizoside**(40)**	Stem bark	[90]
Molliside**(41)**	Bark	[91]
Grandibracteosides **(42)**	Leaves	[92]



29 **30** **31**



32 **33**



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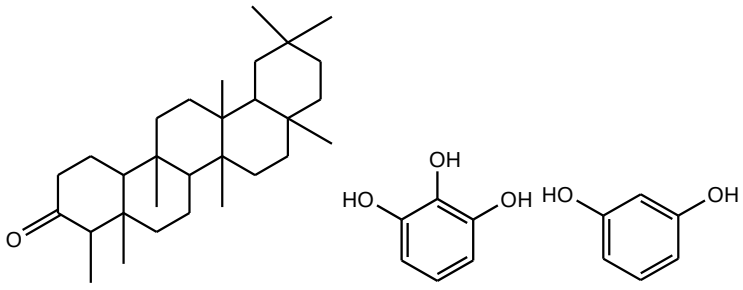


36 **37** **38**

Figure 7: Some of the chemical structures of the isolated compounds from the genus *Albizia*

2.2.6 Biological activities of different *Albizia* species

2.2.6.1 Anti-inflammatory and analgesic activity

The aqueous ethanolic extract of *Albizia amara* roots exhibited significant anti-inflammatory effect in rats atdose of 200 mg/kg administrated compared to the standard dose ofaspirin (100 mg/kg). The anti-inflammatory effect was evaluated usingcarrageenan- induced paw oedema where the percentage inhibition ofoedema was 61.91% [93]. Moreover, it was reported that the aqueous andethanolic extracts of*Albizialebbeck* leaves revealed analgesic effect at doses of 50, 100, and 200 mg/kg administrated to rats. The analgesic effect was evaluated using the hot plate test and tail flick method [94].

2.2.6.2 Antimicrobial activity

An aqueous ethanolic (70%) extract of *Albizia ferruginea* stem bark and leaves showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger,* and *Penicillium notatum.* The anti-microbial activity was evaluated by calculating zoneof inhibition were the leaves extract was more active and *Pseudomonas aeruginosa* was resistant to both extracts [95].

2.2.6.3 Antispermatogenic activity

Oral administration of 50 mg/kg of a saponin-rich fraction obtained from the *Albizia lebbeck* stem bark for 60 days to male rats led to decrease in the weights of testes, epididymides, seminal vesicle and ventral prostate also the production of round spermatid was reduced by 73.04% in the study [96].

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Chemicals and instrumentation

Chemicals and reagents used includes petroleum ether, ethanol, *n*-hexane, chloroform, ethyl acetate, methanol, sulphuric acid, sodium hydroxide, potassium hydroxide, hydrochloric acid, dimethyl sulfoxide, silica gel (70-230 mesh). Melting point wasmeasured using melting point apparatus (MFB 590 010T). NMR spectra, AVANCE II wasprocessed using Bruker 400 spectrometer, using the residual solvent peaks as reference. The spectra were processed using AVANCE II 400 MHz Bruker NMR in $CDCl_3$ solvent. IR was obtained on a FTIR Perkin Elmer spectrometer. TLC analyses were carried out on Merck pre-coated silica gel 60, F_{254} plates. Prep-TLC was done on a glass plates of 20 x 20 cm dimension, pre-coated with silica gel 60, F254. Standard antibiotic drug (*Gentamacin),* antifungal drug (*Mancozeb*), Mueller Hinton agar, Petri dishes, DMSO, plant extracts and Whatman filter paper were used during antimicrobial test.

3.2 Collection and preparation of plant materials

The leaves of *Melia azedarach* was collected from home garden of Tongo town, Mao-Komo special District in Benishangul Gumuz Regional State, Western Ethiopia. Whereas the stem bark of *Albizia schimperiana*was collected from Jimma town, Ginijo Guduru kebele in OromiaRegional State,South Western Ethiopia. Both plants were identified at the Herbarium of the Department of Biology, Addis Ababa University(voucher number 316 and 341, (ETH)) respectively.The collected fresh plant materials were cleaned properly and then allowed to air dry under shade at room temperature. After well drying, the plant materials were ground into a powder with mechanical grinder and stored in a suitable airtight.

3.3 Extraction of plant materials

1 kg of the powdered leaves of *Melia azedarach* and 800 g sample of stem bark of *Albizia schimperiana* were separately soaked in 4 L $CHCl_3$/MeOH (1:1) each twice for 48 hours at room temperature[97]. The extracts obtained were then filtered and concentrated using Rotary Evaporator (Laborata - 4000) at 65 ^{0}C to get the dried crude extracts of 48 g and 34g, respectively.

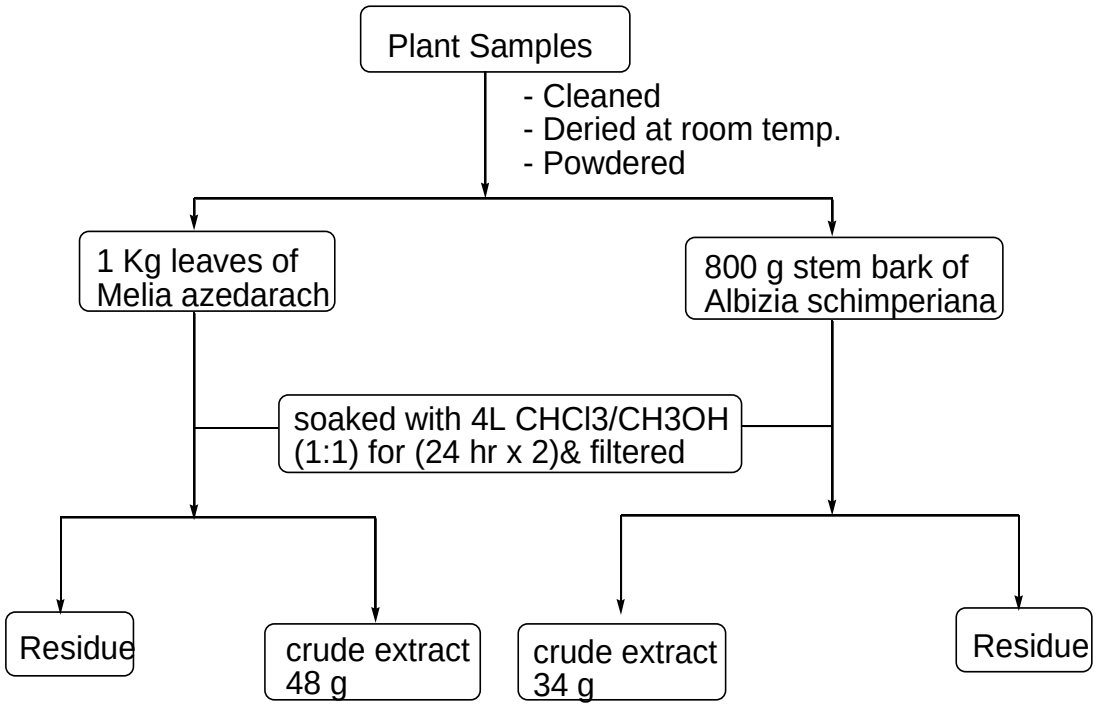


Figure 8: General procedures followed in the extraction of *Melia azedarach* and *Albizia schimperiana*

3.4 Preliminary phytochemical screening

The preliminary qualitative phytochemical screening of the leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana* were performed for testing the presence of different chemical groups such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroid and volatile oil [98-103]in $CHCl_3$/CH_3OH (1:1) extracts.

I. Test for alkaloids

Wagner's Test: A fraction of extract was treated with Wagner's test reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and the formation of a reddish brown color indicated the presence of the alkaloids [98].

II. Test for flavonoids

Sodium Hydroxide Test: Plant extract is treated with dilute NaOH, followed by addition of dilute HCl. A yellow solution with NaOH turns colorless with dilute HCl, which shows the presence of flavonoids [99].

III. Test for phenols and tannins

The plant extract was diluted with water and 3-4 drop of 10% ferric chloride solution was added. Appearance of the blue-green or black color indicated the presence of phenol and tannins [100].

IV. Test for saponins

2ml of extract was taken and treated with hot water and vigorously shaken for 30 sec. Thick froth was formed which confirmed the presence of saponins [101].

V. Test for terpenoids

To conduct this, 5ml of plant extract is added to 2ml of chloroform and 3ml of concentrated sulphuric acid. The presence of terpenoids gives a reddish brown color of interface [102].

VI. Tests for steroid

Salkowski reaction:A few crystals of compounds 1 and 2 were dissolved in chloroform in different test tube and a few drops of concentrated sulphuric acid were added to the solution, both compounds 1 and 2 formed a reddish color in the upper chloroform layer [103] indicating presence of steroids.

3.5Antimicrobial activity test

3.5.1 Preparation of test solutions

The test solution of both plants were prepared individually by dissolving 50 mg of each crude extracts and 20 mg of the isolated compounds in 1 mL of dimethyl sulfoxide (DMSO) to prepare 50 mg/mL and 20 mg/mL stock solution of the test samples respectively. The standard drug for antibacterial taste (*Gentamacin* 10mg/ml) and antifungal taste (*Mancozeb*10mg/ml).The culture media was prepared by dissolving 6.08 g of Muller Hinton Agar in 160 mL of distilled waterand boiled to dissolve the media completely.

3.5.2Preparation microbial cultures

The activity of the plant extracts were tested against four bacterial strains and two funguswhich are disease causing infectious in living organism, two Gram-positive bacteria (*Bacillussubtilis*and *Staphylococcus aureus)*and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli)*and two fungus i.e. *Aspergillus flavus* and*Fusariumspp.*were used to evaluate antimicrobial activities. These standard bacterial strains were obtained from EPHI and preserved until usedin the Department of Biology, Jimma University whereas, the fungus were obtained from theDepartment of Biology, Jimma University.

3.5.3Antimicrobial assay

(a) Antibacterial assay

The antibacterial activity test was done using disc diffusion method standard procedures [104] to test the extracts and isolated compounds against the bacteria strains. Muller Hinton Agarculture media was used for growing of organisms whereas *Gentamacin* was used as standard drug. The culture media was prepared using distilled water and boil to dissolve the media completely and sterilized by autoclaved at 121^0C for 2 hours, then poured into sterile Petri dishes under sterile conditions. After the culture media was solidified, then 1mL of bacterial suspension was uniformly added to it. Filter paper pieces

containing the test sample were put on Petri dish and then finally incubated at 37^0C for 24 hours. After overnight incubation, the diameter of inhibitory zone formed around each discs were measuredusing ruler in mm and the observed results wasrecorded.

(b) Antifungal activities test

A disc diffusion method was applied to test the plants extracts against the tested fungus [105] using standard antifungal agent *Mancozeb* as a positive control. The prepared culture mediawas autoclaved for 2 hours at 121^0C temperature. After the culture media was solidified, then 1mL ofthe fungal solutions were uniformly added to it. Filter paper pieces containing the test sample were put on Petri dish and then the Petri dishes was covered and incubated at 27^0C for 72 hrs. DMSO solvent was used as a negative control for each Petri dish. Finally, the results were taken on the third day by measuring the diameter of zone of inhibition.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Isolation and identification of compounds

The air dried and powdered leaves sample of*Melia azedarach* and stem bark of *Albizia schimperiana*were separately soaked using chloroform/methanol (1:1, v/v), the obtained result of the crude extracts weighed 48 g and 34g respectively. The crude extract of the leaves of *Melia azedarach* (15 g) and the stem bark of *Albizia schimperiana* (30 g) were separately subjected to column chromatography on silica gel(300 g) and eluted with the mixture of petroleum etherand ethyl acetate with increasing polarities.

4.1.1 Compounds isolated from the leaves of *Melia azedarach*

The column was first eluted with petroleum ether as the mobile phase by increasing polarity by 2 % increments of ethyl acetate up to 100 % ethyl acetate to provide 128 fractions (50 ml each). The collected fractions were concentrated to dryness using a rotary evaporator at 65^0C and were subjected to TLC analyses. **MA 1**,colorless needle (50 mg) was obtained from the coloumn fraction number 57-61 eluted with 15% ethyl acetate in petroleum ether (Rf- value 0.52 in petroleum ether /ethyl acetate,75:25) and its melting point is 138^0C-140^0C. **MA 2**, a redish crystalline solid (40 mg) was collected from the coloumn fraction number 97-107 eluted with 25% ethyl acetate in petroleum ether (Rf- value 0.39 in petroleum ether - ethyl acetate, 75:25) and its melting point is180^0C -186^0C.

NB: uncharacterized compounds are simply labeled by a lab. Code.

4.1.2 Compounds isolated from the stem bark of *Albizia schimperiana*

Similarly, the column was first eluted with petroleum ether as the mobile phase by increasing polarity by 2 % increments of ethyl acetate up to 100 % ethyl acetate toprovide 185 fractions (50 ml each) to afford eight compounds of different purity level. **Asc 1**, colouless crystalline solid (38 mg) was collected from the coloumn fraction

number 1- 20 eluted with 100 % of petroleum ether. The Rf- value of this compound was determined to be 0.56 in petroleum ether/ethyl acetate (90:10) and its melting point is 134 ^{0}C-136 ^{0}C.Whereas, the fractions collected with 8% ethyl acetate in petroleum ether (Fr. 67-75) afforded **Asc 6** with Rf value 0.41 and its melting point is 103^{0}C-105^{0}C.

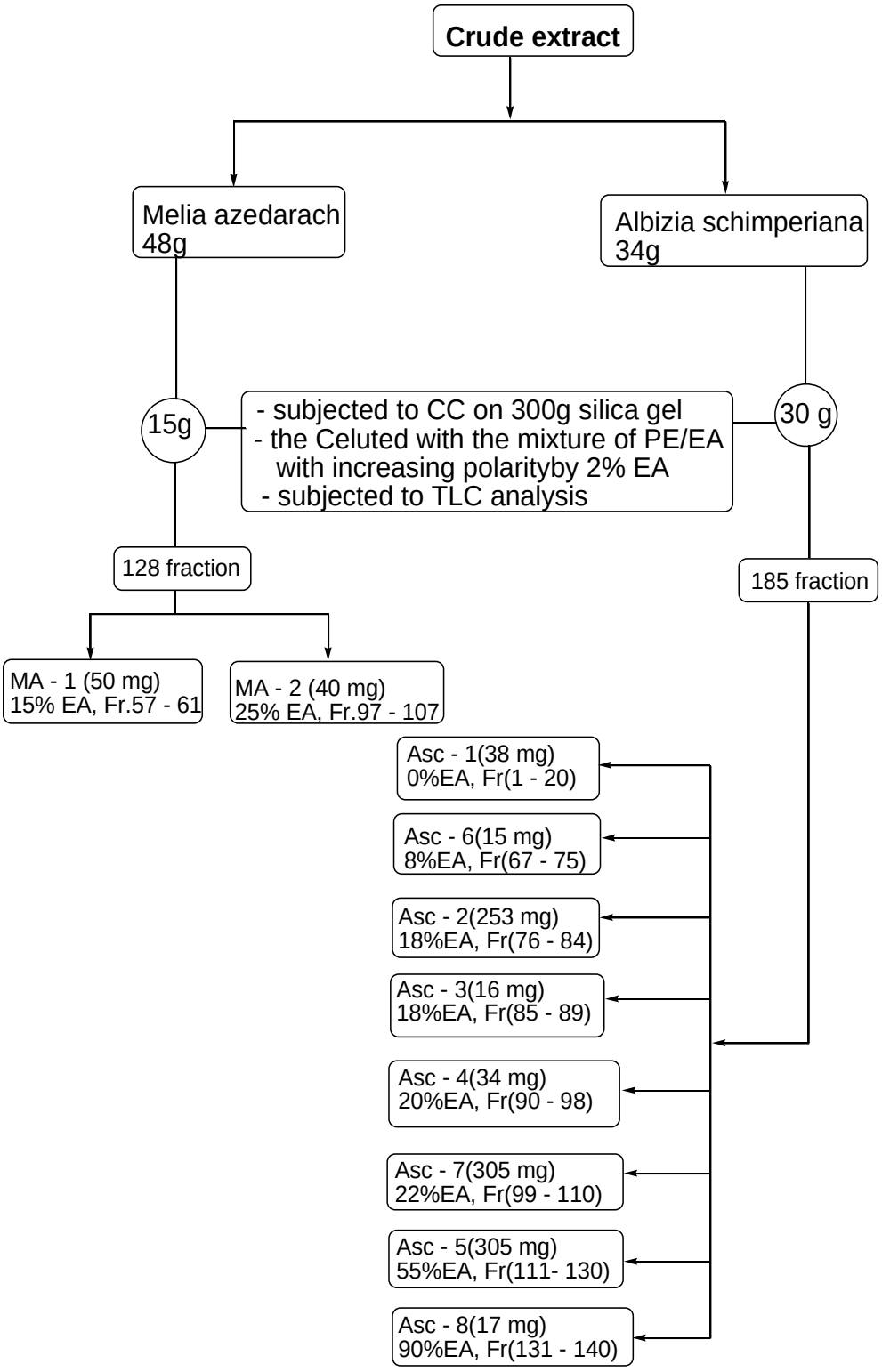


Figure 9: General procedures followed in the extraction and isolation of compounds from the leaves of *Melia azedarach*and stem bark of *Albizia schimperiana.*

Two compounds were isolated from the leaves of *Melia azedarach*and eight compounds from the stem bark of *Albizia schimperiana*. Only two compounds were characterized from both plants in this Thesis which are leveled as **MA 1** and **Asc 1**. There are also some other impure compounds which will be purified well and to be characterized in near future.The Chemical study of the leaves extract of *Melia azedarach* andstem bark of *Albizia schimpiriana* afforded two pure compounds whosestructures were established as *β*-sitosterol and *α*-spinasterol respectively by extensive spectroscopic studies and direct comparison of their spectrum with published data.

MA 1Asc 1

Figure 10: The two characterized compounds from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana*.

Some of the physical properties of the two characterized compoundsare mentioned us follows

Physical properties	**MA 1**	**Asc 1**
Color	Colorless needle sub.	Colorless crystalline solid
Mass	50mg	34mg
Fraction number	57-61	1- 20
Polarity ratio	15% EA	0%
Melting point	138^0C - 140^0C	134^0C - 136^0C
Rf. value	0.52	0.56

4.2Preliminary phytochemical screening

The preliminary phytochemical screening of both plants showed the presence of various secondary metabolites and the result of phytochemical test has been summarized in (Table 4).

Table 4: Bioactive components of *Melia azedarach*leaves extract and*Albizia schimperiana* stem bark extract ($CHCl_3$/CH_3OH).

Phytochemicals	***Melia azedarach***	***Albizia schimperiana***
Alkaloids	+	+
Flavonoids	-	+
Phenols	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Steroid	+	+

NB: +sign indicate the presence of phytochemical constituents.

-Sign indicate the absence of phytochemical constituents.

4.3 Structural elucidation of the isolated compounds

4.3.1 Spectroscopic data of the isolated compounds

MA 1:^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm):5.34 (1H, d, H-6), δ 3.51 (1H, m, H-3), δ 1.16, δ 1.26 (3H, s, H-19 and H-18), δ 0.94 (3H, d, H-21), δ 0.85 (3H, t, H-29), δ 0.83 (3H, d, H-26) and δ 0.83 (3H, d, H-27). ^{13}CNMR (100 MHz, $CDCl_3$) δ (ppm): 37.3 (CH_2, C-1), δ 33.9 (CH_2, C-2), δ 71.8 (CH, C-3), δ 42.2 (CH_2, C-4), δ 140.7 (C, C-5), δ 121.7 (CH, C-6), δ 29.2 (CH_2, C-7), δ 31.7 (CH, C-8), δ 50.2 (CH, C-9), δ 36.5 (C, C-10), δ 23.1 (CH_2, C-11), δ 39.8 (CH_2, C-12), δ 42.3 (C, C-13), δ 56. 8 (CH, C-14), δ 24.4 (CH_2, C-15), δ 28.2 (CH_2, C-16), δ 56.1 (CH, C-17), δ 12.0(CH_3, C-18), δ 19.8 (CH_3, C-19), δ 40.5 (CH, C-20), 19.4 (CH_3, C-21), δ 36.2 (CH_2, C-22), δ 26.1, (CH_2, C-23), δ 51.2 (CH,

C-24), δ 31.9 (CH, C-25), δ 21.2 (CH_3, C-26), δ 21.1 (CH_3, C-27), δ 25.4 (CH_2, C-28), 12.2 (CH_3,C-29)

Asc 1:^{1}H NMR ($CDCl_3$,400MHz) δ (ppm) : 5.37 (1H, t, H-7), 5.17 (1H, d, H-22), 5.16 (1H, d, H-23), 3.52 (1H, m, H-3), 1.27 (3H, d, H-21), 1.03 (3H, d, H-26), 1.02, (3H, d, H-27), 1.30 (3H, s, H-19), 0.95 (3H, t, H-29), 1.33 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) : 37.3 (C-1), 31.5 (C-2), 71.1 (C-3), 38.0 (C-4), 40.3 (C-5), 29,7 (C-6), 117.5 (C-7), 139.6 (C-8), 49.4 (C-9), 34.2 (C-10), 25.4 (C-11), 39.5 (C-12), 43.3 (C-13), 55.9 (C-14), 23.0 (C-15), 29.6 (C-16), 55.1 (C-17), 12.0 (C-18), 19.0 (C-19), 40.9 (C-20), 21.1 (C-21), 138.2 (C-22), 129.4 (C-23), 51.2 (C-24), 31.7 (C-25), 21.5 (C-26), 21.4 (C-27), 25.4 (C-28) and 12.3 (C-29).

4.3.2 Structural elucidation of MA 1 from leaves extract of *Melia azedarach*

MA 1, was isolated as a white crystalline solid with melting point 138-140^{o}C. The elemental analysis of *β*-sitosterolindicated that the compound contains 83.86 % of C, 12.25 % of H and 3.89 % of O, the molecular weight could be either 411.30 ($C_{29}H_{50}O$) or 822.62 ($C_{58}H_{100}O_2$), respectively. From ^{13}C NMR and ^{1}H NMR spectra, the number of C and H was found to be near to $C_{29}H_{50}O$. This formula produces 239 hits when searched in http://www.chemspider.com/Search.aspx. Since, the compound gives positive test for steroid, all of the other structures other than steroid were rejected. The general formula for the compound was C_nH_{2n-8} [106].

The ^{1}H NMR spectroscopic data of **MA 1**, (Figure 13) showed the presence of six methyl signals that appeared as two methyl singlet's at δ 1.26 and δ 1.16, assignable for H-18 and H-19 respectively; three methyl doublets that appeared at δ 0.83, δ 0.83, and δ 0.94 for H-27, H-26 and H-21, respectively and a methyl triplet at δ 0.85 for H-29. The spectrum also displayed a proton corresponding to the C-3 hydroxy group, which appeared as a multiplet at δ 3.51 andone olefin proton at δ 5.34 ppm.

The ^{13}C NMR spectroscopic data of **MA 1**,(Figure 14) showed signals for 29 carbon atoms including an oxymethine carbon signal at δ 71.8 and two olefin carbons at δ 140.7 and δ 121.7 ppm. The double bonded carbon appeared at δ 140.7 and δ 121.7 ppmwere

assigned for the olefin carbons C-5 and C-6and two methylene carbon signals were exhibited at δ 36.2 and δ 26.1 ppm for C-22 and C-23. In addition, the spectrum showed a signal at δ 71.8 for C-3 *β*-hydroxyl group, three up field chemical shifts at *δ* 19.8, 12.0 and 19.4, respectively for C-18, C-19 and C-21 position. The downfield signals at *δ* 31.7, 36.5 and 56.8 were assignable to the carbon at positions C-8, C-10 and C-14. From DEPT-135 (Figure 15), it confirmed that this compound is having six methyl (CH_3) groups, eleven methylene (CH_2) groups, nine methine (CH) groups and three quaternary carbons. The physical and spectral data of the isolated compound was in good agreement for the structure of *β*-sitosterol having a molecular formula of $C_{29}H_{50}O$ by direct comparison of its spectrum with the reported data in literature value [107, 108]. Based on the spectroscopic data and comparison with literature, **MA 1**was identified as *β*-sitosterol with a molecular formula of $C_{29}H_{50}O$(Figure 11). The compound further confirmed by comparing its NMR spectrum data with those reported in previous studies [109, 110].

Table 5: Chemical shifts of ^{1}H NMR and ^{13}CNMRof **MA 1** with the reported data in literature [111, 112].

Carbon atom	**^{1}HNMR Experimental**	**^{1}HNMR Literature**	**^{13}CNMR Experimental**	**^{13}CNMR Literature**	**Nature of carbon**
C-1	1.47	1.47	37.3	37.3	CH_2
C-2	1.56	1.56	33.9	31.7	CH_2
C-3	3.51	3.52	71.8	71.8	CH
C-4	2.28	2.28	42.2	42.3	CH_2
C-5	-	-	140.7	140.7	C
C-6	5.34	5.36	121.7	121.7	CH
C-7	2.03	2.03	29.2	31.7	CH_2
C-8	1.45	1.67	31.7	31.9	CH
C-9	1.44	1.48	50.2	50.2	CH
C-10	-	-	35.5	36.5	C
C-11	1.52	1.52	23.1	21.1	CH_2
C-12	1.49	1.49	39.8	39.8	CH_2
C-13	-	-	42.3	42.3	C
C-14	1.40	1.50	56.8	56.8	CH
C-15	1.35	1.60	24.4	24.4	CH_2
C-16	1.60	1.84	28.2	28.2	CH_2
C-17	1.47	1.49	56.1	56.1	CH
C-18	1.26	0.68	19.8	11.9	CH_3
C-19	1.16	1.02	12.0	19.4	CH_3
C-20	1.64	1.64	40.5	36.5	CH
C-21	0.94	0.94	19.4	18.8	CH_3
C-22	0.88	0.88	36.2	34.0	CH_2
C-23	1.25	1.04	26.1	26.1	CH_2
C-24	1.46	1.50	51.2	45.9	CH
C-25	1.82	1.65	31.9	28.9	CH
C-26	0.83	0.83	21,2	19.8	CH_3
C-27	0.83	0.85	21.1	18.8	CH_3
C-28	1.29	1.04	25.4	23.1	CH_2
C-29	0.85	0.88	12.2	12.0	CH_3

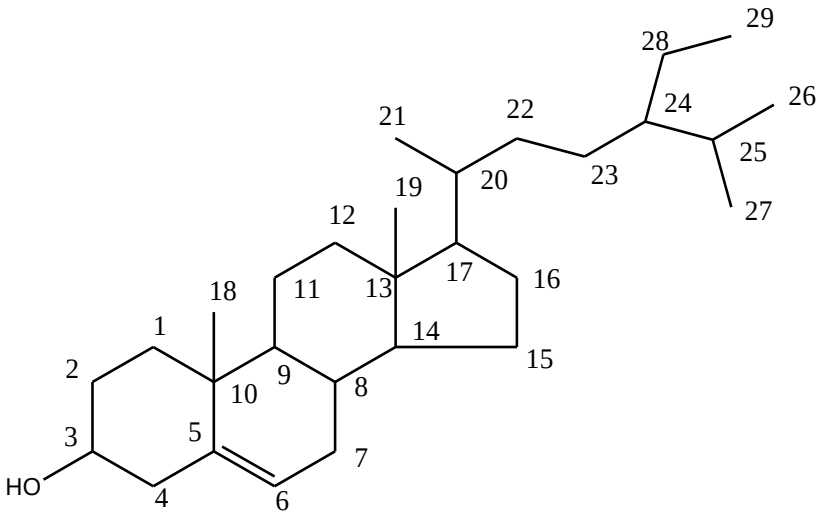


Figure 11: Structure of β-stiosterol from the leaves extract of *Melia azedarach*

4.3.3 Structural elucidation of Asc 1 from stem bark extract of *Albizia schimperiana*

Sterols belong to a large group of hydrocarbons, phytosterols which have in common derivatives of a tetracyclic perhydro-cyclopentano-phenanthrene ring system with a flexible side chain at the C-17 atom and 3*β*-monohydroxy compounds [113], sometimes bearing methyl groups at C-10 and C-13. The structure ofthe isolated**Asc 1**was characterized with the help of spectroscopic methods (IR and 1D NMR) in the sub-sections below.

Asc 1isolated as colorless crystalline solid with melting point 134°C-136°C. On subjection to IR spectroscopic analysis (Figure 16), band was observed at 3407 cm^{-1} that is characteristic of -OH stretching. Absorption at 2917 cm^{-1} is due to aliphatic -CH stretching. Other absorption frequencies include 1631 cm^{-1} as a result C=C stretching and weak band, at 1473 cm^{-1} is a bending frequency for cyclic $(CH_2)n$, and 1384 cm^{-1} for -$CH(CH_3)_2$. The absorption frequency at 1063 cm^{-1} signifies cycloalkane. The out of plane -CH vibration of unsaturated part was observed at 719cm^{-1}. The ^1H NMR spectroscopic data of **Asc 1**, (Figure 17) varied between 0.95 to 5.37 ppm. This spectrum showed the presence of 6 high intensity peaks indicating the presence of six methyl groups at δ 0.95, 1.02, 1.03, 1.27, 1.30 and 1.33 ppm for H-29, H-27, H-26, H-21, H-19 and H-18, respectively. The proton corresponding to the H-3 of a spinasterol moiety was appeared

as a multipletat δ 3.52 ppm. Two olefin protons appeared at δ 5.17 (1H, dd) and 5.16 (1H, dd) in the ^{1}H NMR spectrum for H-22 and H-23, respectively.

The ^{13}C NMR spectroscopic data of **Asc 1**,(Figure 18) gave signal for olefin carbon at 117.5 and 139.6 ppm for C-7 and C-8, respectively, 71.1 for C-3 attached to a hydroxyl group, 19.0 and 12.0 for angular methyl carbon atoms for C-18 and C-19, respectively. The chemical shift at δ 37.3, 31.5 71.1, 38.0, 29.7, 117.5, 25.4, 39.5, 23.0 and 29.6 ppm were appropriate for the cyclohexyl and cyclopentyl carbon atoms at positions C-1, C-2, C-3, C-4, C-6, C-7, C-11, C-12, C-15 and C-16, respectively. The other shifts at δ 40.9, 51.2 and 31.7 ppm were assigned for the carbon numbers C-20, C-24, and C-25, respectively, which constitute the side chain of three carbons which were linked at position 17 of the cyclopentyl ring. Furthermore, the chemical shift at δ 55.1 ppm was assigned for the carbon number C-17 which was the point of link of a side chain to the cyclopentyl ring. The alkenes' carbons appeared at δ 117.5, 139.6, 138.2 and 129.4, for C-7, C-8, C-22 and C-23, respectively. From the spectral data of DEPT-135(Figure 19) it indicated that this compound is having four olefin carbons, one oxygenated carbon, seven methine carbons, two quaternary carbons, nine methylene carbons, and six methyl carbons. These are characteristic resonances of a sterol with an alcohol and two olefin bonds. On the basis of IR, ^{1}H NMR, ^{13}C NMR, DEPT-135 spectral dataand the other physical properties, the isolated compound was in good agreement for the structure of α-spinasterol having a molecular formula of $C_{29}H_{48}O$ [114]. Therefore **Asc 1**was identified as α-spinasterol(Figure 12).The NMR spectrum of this compound resembled to the data published in previous studies [115]. This is the first report of isolation of α-spinasterol from the species of *Albizia schmiperiana*but not for the genus *Albizia*.

Table 6: Chemical shifts of ^{1}H NMR and ^{13}CNMR of **Asc 1**with the reported data in literature [114].

Carbon atom	**^{1}HNMR Experimental**	**^{1}HNMR Literature**	**^{13}CNMR Experimental**	**^{13}CNMR Literature**	**Nature of carbon**
C-1	1.50	1.09, 1.82	37.3	37.2	CH_2
C-2	1.54	1.39, 1.77	31.5	31.5	CH_2
C-3	3.52	3.59	71.1	71.1	CH
C-4	1.22	1.27	38.0	38.0	CH_2
C-5	1.45	1.40	40.3	40.3	C
C-6	1.76	1.22, 1.74	29.7	29.7	CH
C-7	5.37	5.15, s	117.5	117.5	CH_2
C-8	-	-	139.6	139.6	CH
C-9	1.93	1.65	49.4	49.5	CH
C-10	-	-	34.2	34.2	C
C-11	1.48	1.48	23.4	21.6	CH_2
C-12	1.41	1.22, 2.02	39.5	39.6	CH_2
C-13	-	-	43.3	43.3	C
C-14	2.17	1.81	55.9	55.1	CH
C-15	1.62	1.40, 1.52	23.0	23.0	CH_2
C-16	1.39	1.25	29.6	28.5	CH_2
C-17	1.51	1.25	55.1	55.9	CH
C-18	0.95	0.55, s	19.0	12.0	CH_3
C-19	1.02	0.80, s	12.0	13.0	CH_3
C-20	2.33	2.05	40.9	40.8	CH
C-21	1.27	1.03d (6.8)	21.1	21.4	CH_3
C-22	5.17	5.16dd (8.8,15.2)	138.2	138.1	CH_2
C-23	5.16	5.02dd (8.4, 15.2)	129.4	129.5	CH_2
C-24	2.15	1.55	51.2	51.2	CH
C-25	1.86	1.55	31.7	31.9	CH
C-26	1.03	0.85d (6.4)	21.5	21.1	CH_3
C-27	1.02	0.84d (6.0)	21.4	19.0	CH_3
C-28	1.33	1.18, 1.42	25.4	25.4	CH_2
C-29	0.85	0.81t (7.2)	12.3	12.2	CH_3

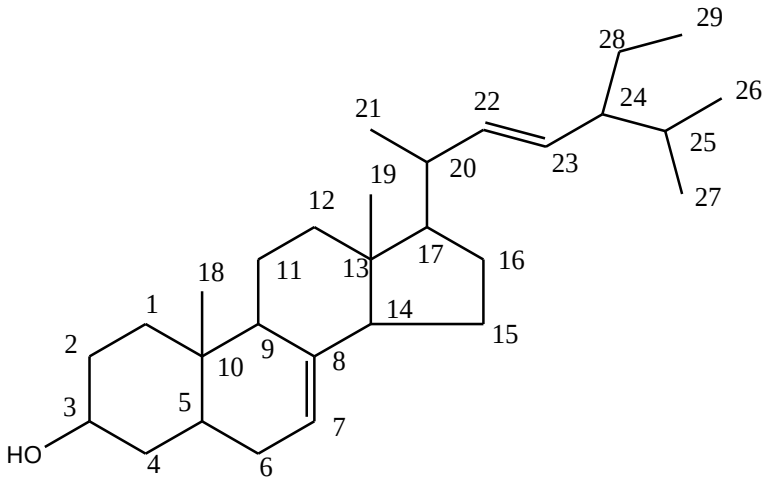


Figure 12: Structure of α-spinasterol from the stem bark of *Albizia schimperiana*

4.4Antimicrobial activity data

In the present investigation, the crude extracts and isolated compounds of both plantswere evaluated for exploration of their antimicrobial activity against four bacterial strains and two fungi species which were regarded pathogenic microorganism. The antimicrobial activity of each sample was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with the ruler and the results of the activity was recorded. The effectiveness of the samples were also examined among each other against the tested pathogens by comparing the maximum zone of inhibition.

4.4.1 Antibacterial activitytest resultsdata

The preliminary qualitatively investigation showed that all samples were active against all the tested bacteria strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*and *Pseudomonas aeruginosa)* except the crude extract of *Albizia schmiperiana* which was only active against two bacterial strains *(Escherichia coli* and *Bacillus subtilis).* The obtained results were assessed quantitatively on the basis of inhibition zone and summarized in (Table 8).

Table 7: Antibacterial activity of the test samples with the standard drug solution**(Gentamacin)**

Test sample	Conce. mg/mL	Diameter of zone inhibition of each tested organism (mm)			
		Escherichia coli (-)	*Pseudomonas aeruginosa* (-)	*Staphylococcus aureus* (+)	*Bacillus subtilis* (+)
MA 1	20	14.2	22.8	15.5	19.9
Asc 1	20	12.4	14.7	13.0	16.0
MA crude	50	8.0	10.4	8.3	10.2
Asc crude	50	7.8	NI	NI	8.0
Gentamacin	10	20.4	40	30	28
DMSO		NI	NI	NI	NI

NB-Gram-negative bacteria

+ Gram positive bacteria

MA 1β-stiosterol

Asc 1α-spinasterol

NINot inhibited

MAMelia azedarach

Asc Albizia schimperiana

MA1 (β-stiosterol)has shown more effective antibacterial activities than that of compound 2awith the comparison of the standard drug (Gentamacin) as demonstrated by the observed inhibition zone values (Table 8). The obtained results also indicated that **MA 1**(β-stiosterol)exhibit relatively higher zone of inhibition against two bacterial strains namely,*Bacillus subtilis*(19.9mm) and *Pseudomonas aeruginosa* (22.8mm)with the comparison of standard drug Gentamacin (28mm and 40mm) respectively. In addition, the analysis of antibacterial activity test indicated that the crude extract of *Melia azedarach* have better impact on all the tasted species of pathogenic bacteria when compared to crude extract of *Albizia schimperiana.* From the results, it is clear that the medicinal values of the leaves extract of *Melia azedarach* are more effective than that of the stem bark of *Albizia schimperiana*against tested antibacterial activities. On the other

hand, the crude extract of *Albizia schimperiana* was not active against two tested bacteria strains namely, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The overall observation indicated that the crude extract and isolated compounds of *Melia azedarach* exhibit relatively higher zone of inhibition compared to the crude extract and isolated compounds of*Albizia schimperiana*.

4.4.2 Antifungal activities test data

The fungus used for the test were (*Aspergillus flavus* and*Fusarium spp.*), the results of antifungal activity of both plants against the investigated funguswere assessed quantitatively on the basis of inhibition zone and shown in (Table 9).

Table 8:Antifungal activity of the test samples with the standard drug solution***(Mancozeb)***.

Test sample	**Concentration**	**Diameter of zone inhibition of each tested organism (mm)**	
	mg/mL	*Aspergillus flavus*	*Fusarium spp.*
MA 1	20	12.3	13.2
Asc 1	20	10.0	10.7
MA crude	50	8.1	7.5
Asc crude	50	8.0	NI
Mancozeb	10	14.6	18.0
DMSO		NI	NI

NB-Gram-negative bacteria

+ Gram positive bacteria

MA 1β-stiosterol

Asc 1α-spinasterol

NINot inhibited

MAMelia azedarach

Asc Albizia schimperiana

The observedresults showed that all samples were active against the tested fungus (*Aspergillus flavus* and*Fusarium spp.*) except the crude extract of *Albizia schmiperiana* which was active only against *Aspergillus flavus*.Similar to antibacterial activity test, the collective analysis of antifungal activity of the crude extract as well as the isolated compound of *Melia azedarach* have better impact on both the tested species of pathogenic fungal when compared to crude extract and the isolated compound of *Albizia schimperiana*by the observed inhibition zone values (Table 9). The phytochemicals of both plants belongs to the chemical classes of Phytosterol. These chemicals are characterized for their antimicrobial activities [116].

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Medicinal plants are rich sources of a wide variety of chemical compounds and have been used as a major constituents of most indigenous medicines for a variety of diseases.The preliminary phytochemical screening of both plants showed the presence of different chemical groups such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroid and volatile oil. *Melia azedarach* and *Albizia schmiperiana*plants are one of the potential medicinal plants used for the treatment of various diseases caused by microorganisms.Thepresent studyresulted in the characterization of two pure compounds, the first compound was isolated from the leaves of *Melia azedarach* has been characterized as *β*-stiosterol and the second compound from stem bark of *Albizia schmiperiana* identified as *α*-spinastestrol. In the study, the isolated compounds as well as the crude extract of both plantswere evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria strains and fungi species. The obtained result demonstrates that the isolated compounds of both plants possess strong /significant/ inhibitory effect against tested pathogens than the crude extract. In comparison to both plants, the crude extract as well as the isolated compound of *Melia azedarach* inhibited the growth of the testedfour bacterial strains and two fungal more effectively.On the other hand,the crude extract of *Albizia schmiperiana*showed minimum level of inhibition zone against the tested bacterial strains and fungus used in the present study. The overall observation indicated that the leaves extract of *Melia azedarach* has relatively active medicinal values than that of the stem bark of *Albizia schmiperiana*. The scientific findings of the present study support the folklore claim along with the therapeutic application of both plants against microbial activitiesfrom *in vitro* assay result and an important step towards their acceptance and development of modern drugs.

Generally, even if the efficacy of both plants against microbial activity were verified in the present work, their toxic level towards human health was not examined.Therefore,

further studies are needed to test their toxicity level and looking toward a pharmaceutical use.

5.2 Recommendations

- The present study simplyfocused on the evaluation of antimicrobial activities of the leaves of *Melia azedarach* and the stem bark of *Albizia schimperiana* medicinal activities, it would be recommended for the determination of MICvalues provides a quantitative measure for the level of resistance expressed by the test organism.
- There is need for conducting more studies to identify and characterize the chemical principles in the tested plants, which may serve as novel compounds for development of new and more effective antimicrobial activities.
- Investigation of other parts of the same plants to establish their activities against other infection diseases are recommended.

6. REFERENCES

[1] Abdullahi, A.A. Trends and challenges of traditional medicine in Africa. *Afr. J. Trad. Complem. Alte. Med*. **2011**, *8*, 115–123.

[2] WHO. General Guidelines for Methodologies on Res. and Evaluation of Traditional Medicine WHO, Geneva, Switzerland, 2000.

[3] Bodeker, G.Traditional health knowledge and Public policy. Nature and Resource.1994,30, 5-16.

[4] Cardini, F.; Wade, C.; Regalia, AL. Clinical research in traditional medicine: priorities and methods. Complem. *Alte. Med*. **2006**, *14*, 282–87.

[5] Davidson-Hunt I. Ecological ethno botany: stumbling toward new practices and paradigms. *MASA J*.**2000,** 161–13,

[6] Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K. M.; Latha, L. Y. Extraction, Isolation a Characterization of Bioactive Compounds from Plants' Extracts. Afr. J. Traditional, Complem., Alter. med., **2011**, *8*, 1-10.

[7] WHO.*Res. Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines*. Manila. 1993.

[8] UNESCO. *Culture and Health, Orientation Texts World Decade for Cultural Development*, 1996, pp129.

[9] Geyid, A.; Abebe, D.; Debella, A.; Makonnen, Z.; Aberra, F.; Teka, F.; Kebede, T.; Urga, K.; Yersaw, K.; Biza, T.; Mariam, B. H.; Guta, M. Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. *J. Ethnopharmacol.*,**2005,***97*, 421–427.

[10] Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K. M.; Latha, L. Y. Extraction, Isolation an Characterization of Bioactive Compounds from Plants' Extracts. *Afr. J. Trad., Complem., Alter. med,* **2011**, *8*, 1-10.

[11] Baby, J. Pharmaco.and phytochemical properties of *Aleovera* Linn an overview.*Pharma. Sci. Review and Res.,Int. J.*, **2010**, *4*, 106-110.

[12] Chew, Y. L.; J.K.; GOH and Y.Y.; LIM. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from *Leguminosae* family in Peninsular Malaysia. *Food Chemistry*. **2009**, *119*, 373–378.

[13] Cowan, M.M. *Plant products as antimicrobial agents*.Clinical Microbiology Reviews,1999, 12, 564–582.

[14] Pichersky, E. J.; Gershenzon. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Current Opinion in Plant Biology, 2002,5, 237–243.

[15] Harborne, J.B.;N.J.; Walton, D.E. *Brown Classes and functions of secondary products from plants. Chemicals from Plants*; London, Imperial College Press, *Edn.*1999,1-25.

[16] Singh, S.B.; J.F.; Barrett. Empirical antibacterial drug discovery-foundation in natural products. *Biochemical Pharmaco.*,**2006,***71*, 1006–1015.

[17] AlBari M. A.; Sayeed, M. A.; Rahman, M.S. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis.*A novel species collected in Bangladesh. Res.*J. Med. Sci*.,**2006**, *1*, 77-81.

[18] Sieradzki, K.; Wu, S.W; and Tomasz, A. Inactivation of the methicillin resistance gene mecA in vancomycin-resistant *Staphylococcus aureus*. *Micro. Drug Resist*. **1999**, *5*, 253-257.

[19] Van Der Waaij, D.; Nord, C.E. Development and persistence of multi-resistance to antibiotics in bacteria: An analysis and a new approach to this urgent problem. *Int. J. Antimicrobial Agents.* **2000**, *16,*191-7.

[20] Kameshwara, Rao,C. Database of medicinal plants: Government of Karnataka, India. 2000, pp1-23.

[21] Byarugaba, D.K. A view on antimicrobial resistance in developing countries and responsible risk factors. *Int. J. Antimicrobial. Agents.***2004**, *24*, 105–110.

[22] Abreu, A.C.; Borges, A.; Simões, L.C.; Saavedra, M.J.; Simões, M. Antibacterial activity of phenyl isothiocyanate on *Escherichia coli* and *Staphylococcus aureus*. *Med. Chem.* **2013**, *9*, 756–761.

[23] Hart, CA.; Karriuri, S.Antimicrobial resistance in developing countries *J.*,**1998**, *317*, 421-452.

[24] Suresh, K.; Deepa, P.; Harisaranraj, R.; Vaira, A. V. Antimicrobial and Phytochemical investigation of the leaves of Carica papaya, Cynodondactylon, Euphorbia, Hirta, *Melia azedarach* and Psidiumguajava; Ethnobotanical. **2008**, *12*, 1184-91.

[25] Nahak, G.; Sahu, R.K. In vitro antioxidative activity of *Azadirachtaindica*and*Melia azedarach*leaves by DPPH scavenging assay. *J. Am. Sci*. **2010**, 6, 123-128.

[26] Rubae, A.Y. The Potential Uses of *Melia azedarach*. as Pesticidal and Medicinal Plant, Review. *American-Eurasian J. Sus, Agri*.**2009**, *3*, 185-194.

[27] A. G. Chromophytes of Iran.2nd Ed. Tehran: Tehran University Publication; 1994.

[28] Ramya S, JepachanderamohanP J, Alaguchamy N, Kalayanasundaram M and Jayakumararaj R. In Vitro Antibacterial Prospective of Crude Leaves Extracts of *Meliaazedarach*against *Selected Bacterial Strains. Ethnobot.Leaf*.**2009**, *13*, 254-258.

[29] Watt, G.M.; Brandwijk, B. The Medicinal and Poisonous Plants of Southern and Eastern Afr. E.S. Livingstone Ltd, Edinburg, London. 1962, 745.

[30] Vishnuanta, A.C.; Rana. *Meliaazedarach*: A phytopharmaco. review. *J Pharmaco. Rev.* **2008**, *2*, 173-79.

[31] Ahmad, M.; Qureshi, R.; Arshad, M.; Khan, M. A.; Zafar, M. Traditional herb remedies used for the treatment of diabetes from district Attock (Pakistan). *Pak J Botany*, 2009, *41*, 2777-82.

[32] Schmutterer, H. Properties and potential of natural pesticides from the neem tree, *Azadirachtaindica*. Ann Rev Entomol. 1990, 35, 271-97.

[33] Bauer, A.W., Kirby, M.D.K., Sherris, J.C., Turck, M.Antibiotic susceptibility testing by a standardized single disk method. Am. *J. Clin. Pathol*.*45*, 493–496.

[34] Rao, M.M., Krishna, E.M., Guptam, P.S., Singh,. A new tetranortriterpenoidisolated from the heartwood of Soymida febrifuga. *Ind. J. Chem*. **1978**,*16*, 823–825.

[35] Rao, M.M., Gupta, P.S., Krishna, E.M., Singh, P.; P.Constituents of heartwood of Soymida febrifuga – isolation of flavonoids. *Ind. J. Chem*. **1979**, *17*, 178–180.

[36] Mabberley, D.J. A monograph of *Meliainasia* and the pacific: the history of white cedar and Persian lilac. Gard Bull Singapore. 1984, 37, 49-64.

[37] Nakagawa, H. Duan, H, Takaishi, Y. Limonoids from *Citrus sudachi*. Chem Pharm Bull 2001, 49, 649-51.

[38] N., Garg, H. S., Madhusudanan, K. P. and Bhakuni, D. S. Pant Neem is Healer of All Ailments.*Res. J. of Pharma., Biological and Chemical Sci.*, **2012**, 3, 302–304.

[39] Van der Nat, J. M., Van der Sluis, W. G., Hart, L. A., Van Disk, H., de Silva, K. T. D. and Labadie, R. P., *Planta Med*., **1991**, *57*, 65–68.

[40] Ara, I., Siddiqui, B. S., Faizi, S.; Siddiqui, S., *J. Chem*. *Soc*., *Perkin Trans*., **1989**, *1*, 343–345.

[41] Rastogi, R.P., Mehrotra, B.N., Compendium of Indian Medicinal Plants, CDRI, Lucknow, New Delhi, Publications and Information Directorate.1993, 2.

[42] Siddiqui, B.S., Ghiasuddin, F.S., Rasheed, M. Triterpenoids of the fruit coats of*Azadirachta indica. J. Nat. Prod*. **1999,** *62*, 1006–1009.

[43] Ahn, J.W., Choi, S.U., Lee, C.; O.Cytotoxic limonoids from *Melia azedarach*varjaponica. Phytochemistry.**1994**,*36*, 1493–1496.

[44] Puroshothaman, K.K., Duraiswamy, K., Connolly, J.D., Tetranortriterpenoidsfrom *Melia dubia*. Phytochemistry.1984, 23, 135–137.

[45] Nakatani, M., Shimokoro, M., Zhou, J.B., Okamura, H., Iwagawa, T., Tadera, K.,Nakayama, N., Naoki, H. Limonoids from *Melia toosendan*. *Phytochemistry*, **1999**, *52*, 709–714.

[46] Cantrell, C.L., Rajab, M.S., Franzblau, S.G., Fisher, N.H. Antimyco bacterial triterpenes from *Melia volkensii*. *J. Nat. Prod*. **1999**,*62*, 546–548.

[47] Wu, S.B., Ji, Y.P., Zhu, *J.J., Zhao, Y., Xia, G., Hu, Y.H., Hu, J.F*. Steroids from theleaves of Chinese *Melia azedarach* and their cytotoxic effects on human cancercell lines. Steroids.**2009**,*74*, 761–765.

[48] Sen, A.; Batra, A. Chemical composition of methanol extract of the leaves of *Melia azedarach*. Asian J. Pharma. Clin. Res., 2012,*5*, 42-45.

[49] Dzubak, P, Hajduch M, Vydra D, Hustova A, Kvasnica M, Biedermann D, Pharmaco.Activities of natural triterpenoids and their therapeutic implications. Nat Prod. Rep, 2006, *23*, 394- 411.

[50] Heasley, B. Synthesis of limonoid natural products. Eur J. Org Chem. **2011**, *1*, 19-46.

[51] Joseph-Nathan, P.; Wesener, J.R.; Günther, H. A two-dimensional NMR study of angelic and tiglic acid. *Magn. Res. Chem*. **1984**, *23*, 190-191.

[52] Chiang, C.; Chang, F.C. Tetracyclic triterpenoids from *Melia azedarach*, L.-III. *Tetrahedron* **1973**, *29*, 1911-1929.

[53] Sen, A.; Batra, A. Chemical composition of methanol extract of the leaves of *Melia azedarach*. Asian J. Pharmacol. Clin. Res.**2012**, *5*, 42-45.

[54] Huma, Q., Muhammad, A., Abida A., Naveed I. R., Sammer F.and Muhammad S. A. Ethnopharmaco.and phytochemical account of paradise tree *Melia azedarach,Meliaceae*. *Pure Appl. Biol.,* **2016**, 5, 5-14.

[55] Antara, S.; Amla, B. chemical composition of methanol extract of the leaves of *Melia azedarach.* Asian J. of Pharmaceutical and Clinical Research. *Asian J. Pharm. Clin. Res.*, **2012**, *5*, 1-5

[56] Vishnukanta, A.C.; Rana, A. C. Antifertility activity of *Meliaazedarach* Linn. *Meliaceae* in female Wistar rats. Pharmacology online. **2009**, *2*, 117-132.

[57] Ahmed, M. F.; Rao, A. S.; Ahemad, S. R and Ibrahim, M. Phytochemical Studies and Hepatoprotective activity of *Meliaazedarach* Linn, against CCl_4 induced Hepatotoxicity in rats. *J.Pharmaco.Res.***2012**, *5*, 2664-2667.

[58] Wachsman, M. B.; V, Castilla, C. Inhibition of foot and mouth disease virus (FMDV) uncoating by plant derived peptide isolated from *Meliaazedarach* L. Leaves. Arch. Virod. **1998**, *143*, 581-590.

[59] Alche, L. E.; G. A.; Ferek, M. Meo, C. E.; Coto, M. S. and Maier. An antiviral meliacarpin from leaves of *Meliaazedarach* L. Verlag der Zeitschrift fur Naturforschung Tubingen. **2001**, *58*, 215-219.

[60] Khan, A. V.; Ahmed, Q. U.; Mir MR, Shukla, I, khan A. A. Antibacterial efficacy of the seed extracts of *Meliaazedarach* against some hospital isolated human pathogenic bacterial strains. *Asian Pacific J. Trop. Biomed.* **2011**, *1*,452-455.

[61] Charturvedi, Raseroka, P, Ntshebe, B. H, O. Antimalarial activity of *Melia azedarach*. J App Zoolo Res. **2006**, *17*, 109- 113.

[62] Vidya, V, Srinivasan, Sengottuvelu. Wound healing potential of *Meliaazedarach* L. leaves in alloxan induced diabetic rats. *Global J. Res. Med .Plants Med*. **2012**, *1*, 265-271.

[63] Carpinella, C. M.; Ferraroli, C.; Valladares, G.; Defago, M, Palacios S. Potent limnoid insect antifeedant from *Meliaazedarach*. *Biosci.Biotechnol.Biochem*, **2002**, *60*, 1731 – 1736.

[64] Tewelde, N, Abebe, G.; Eisler, M.; McDermott, J, Greiner, M, et al. *Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia*. Acta Trop.**2004**,*90*, 163-170

[65] King,A., Young, G., *J. the American Dietetic Association*, **1999,** *24*, 213.

[66] Hedberg, I. and Edwards, S. (Editors). Flora of Ethiopia and Eritrea. Addis Ababa, Ethiopia,1989, 3, (96-7, 161-62, 390-92).

[67] Thulin, M.; Fabaceae. In: Hedberg, I.; Friis, I. B. Edwards S (eds.) Flora of Ethiopia,. The National Herbarium, Biology Department, Science Faculty, Addis Ababa University, Ethiopia and the Department of Systematic Botany, Uppsala University, Sweden.1989, 49: 251.

[68] Tariku, Y.In vitro efficacy study of some selected medicinal plants against leishmania spp. MSC thesis paper, Addis Ababa University School of Graduate studies.2008.

[69] Kokwaro J. O.; Johns, T.Medicinal Plants of East Africa. East Afr. Educational Publishers, Nairobi**,**3rd Edn.2013.

[70] Kokwaro, J.O.*Medicinal Plants of East Africa*, 2nd ed. Kenya Literature Bureau, Nairobi, Kenya. Med. Plants of East Afr., 3rd Edition University of Nairobi Press, Nairobi, 2009.

[71] Bekele-Tesemma, A.; Birnie, A.; Tengnäs,B.Useful Trees and Shrubs for Ethiopia. Identification, Propagation and Management for Agricultural and Pastoral Communities. Regional Soil Conservation unit (RSCU), Swedish International Development Authority, Nairobi,1993, 474.

[72] Thulin, M.; Hedberg,F. I.; Edwards, S. Flora of Ethiopia, 3, The National Herbarium. AddisAbaba,1989, 49–251.

[73] Doughari, J. H., A. M. El-Mahmood, I.; Tyoyina. Antimicrobial activity of leaf extracts of Senna obtusifolia. *Afr. J. Pharm. Pharmacol.*,**2008,** *2***,**17-13.

[74] Abdel, K. Taditional use of Albizia. Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry,*Int. J.Advanced Res.*, **2016**,*4*, 1741-1746

[75] Sarkiyayi, S.; Karago, J.; Hassan, R. Studies on Anti Typhoid Properties of Aqueous Methanol Leaves Extract of *Albizia ferruginea (Musase). Int. J. of Biochemistry* Res.& Review.**2011**, *1*, 24-30.

[76] Bobby, Wesely, M. N.; Johnson, E, G, and Antonisamy, M. In vitro antibacterial activity of leaves extracts of *Albizia lebbeck*, Benth against some selected pathogens. Asian Pac. J. of Trop. Biomed.,**2012**, *2*, 859-862.

[77] Kumar, D.; Kumar, S.; Kohli, S.; Arya, R.; Gupta, J.*Antidiabetic activity of methanolic bark extract of Albizia odoratissima Benth*. in alloxan induced diabetic albino mice. Asian Pac. J. Trop. Med.**2011,***4*, 900-903.

[78] Kim, J. H.; Kim, S. Y.; Lee, S. Y.; Jang, C. G. Antidepressant-like effects of *Albizia julibrissin* in mice: involvement of the 5-HT1A receptor system. Pharmacol.Biochem. Behav.2007,87, 41-47.

[79] Cheok, C. Y.; Salman H.; Sulaiman R. Extraction and quantification of saponins: A review. Food Res. Int.2014,5, 16-40.

[80] Uilenberg, G. A. flied guide for diagnosis, treatment and prevention of Afr. animal trypanosomiasis. Food and agricultural organization of the United Nations, 1998.

[81] Rukunga, G.M.; Waterman, P.; G. A new oleanae glycside from the steam bark of *Albizia gummifera. Fitoterapia*.**2001**, *72*, 140-145.

[82] Venkatesh, P., Mukherjee, P.K., Kumav, N.S., Bandyopadhyay, A., Fukui, H., Mizug Vihi, H., Anti-allergic activity of standardized extract of *Albizia lebbeck* with reference of catechin as aphytomarker. *Immunopharm Immunot*. **2010**, *32*, 272-276.

[83] Ganza B. Isolation and Characterization of the Bioactive Compounds in the stem bark of *Albizia coriaria.* 2014, 60-76.

[84] Aparna, V.; Dileep, K.V.; Mandal, P.K.; Karthe, P.; Sadasivan, C.; Haridas, M. Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chem. Biol. Drug Des. 2012, 80, 434–439.

[85] Chen, J.J.; Duh, C.Y.; Chen, I.S. Cytotoxic chromenes from Myriactis humilis. Planta Med. **2005**, *71*, 370–371.

[86] Balogun, O.S.; Oladosu, I.A.; Akiinnusi, A.; Zhiqiang, L. Fatty acid composition -glucosidae inhibitory potential and cytotoxicity activity of Oncoba spinosa Forssk. Elix. Appl. Chem. **2013**, 59, 15637–15641.

[87] Antonisamy, P.; Duraipandiyan, V.; Ignacimuthu, S. *Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from Azima tetracantha Lam. in mouse and rat models.* J. Pharm. Pharmacol. **2011**, *63*, 1070–1077.

[88] Talent, C., Mohammed A.I., Neil A. K. and Shahidul I. *in vitro* antioxidant activities of leaf and root extracts of *Albizia antunesiana* harms. Acta Poloniae Pharma. Drug Res., **2013**, *70*, 1035-1043,

[89] Haddad, M.; Laurens, V.; Lacaille-Dubois M. A. Induction of apoptosis in a leukemia cell line by triterpene saponins from *Albizia adianthifolia*. Bioorg Med. Chem.,2004, 12, 4725-4734.

[90] Liu, R.; Gang, Ma. S, Liu YX, Yu SS, Chen XG, et al. Albizosides D and E, two new cytotoxic triterpenesaponins from *Albiziachinensis*. Carbohydrate Res.,2009, 72, 632-639.

[91] Cheng, Z. Q.; Yang, D.; Ma, Q. Y.; Yi, X. H.; Zhang, N. L. Triterpenoid Saponins from *Albiziamollis*. Bull Korean Chem,2011, 32, 1403-1409.

[92] Krief, S.; Thoison, O.; Sévenet, T.; Wrangham, R.W.; Lavaud, C. Triterpenoid saponin anthranilates from *Albizia grandibracteata* leaves ingested by primates in Uganda. J. Nat. Prod. 2005, 68, 897-903.

[93] Khan, A.; Shah, R.D.; Pallewar, S. Evaluation of Anti-inflammatory and Analgesic Activity of Ethanolic extracts of *Inularacemosa* and *Albizia amara*. *Int. J. of Pharma.and Phytochemical Res*.**2010**, *3*, 22- 27.

[94] Meshram, G.G.; Kumar, A.; Rizvi, W. Tripathi C. D.; Khan R. A. Central analgesic activity of the aqueous and ethanolic extracts of the leaves of *Albizia lebbeck*, role of the Gabaergic and serotonergic pathways. Z Naturforsch ,2015, 70, 25-30.

[95] Agyare, C.; Koffuor, G. A.; Mensah, A. Y.; Agyemang, D. O.Antimicrobial and uterine smooth muscle activities of *Albizia ferruginea* extracts. BoletínLatinoamericano and Caribbean Medicinal Plants and AromáticasMarzo.2006, 5, 31-35.

[96] Gupta, R. S.; Chaudhary, R.; Yadav, R. K.; Verma, S. K.; Dobhal, M. P. Effect of Saponins of *Albizia lebbeck* (L.) *Benth bark on the reproductive system of male albino rats*. J. Ethnopharmacol.**2005,** *96*, 31-36.

[97] Chan, C., G. Ngoh and R. YusofF. A brief review on anti diabetic plants: global distribution, active ingredients, extraction techniques and acting mechanisms. *Pharma. Review*, **2012**, *6*, 22–28.

[98] Lalitha, T.P.; P. Jayanthi. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.)Solms. *Asian J. of Plant Sci. and Res.,* **2012**,*2*, 115-122.

[99] Onwukaeme, D.N., T.B. Ikuegbvweha and C.C. Asonye. Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of *Pycanthus angolensis* Weld Warb (*Myristicaceae*) on corneal ulcers in rabbits.*Tropical J. of Pharma. Res.*,**2007**,*6*, 725–730.

[100] Kikuzaki, H.; Nakatani, N. Antioxidant effects of some ginger con- stituents. *J. Food Sci.* **1993**, *58*, 1407-1410.

[101] Veena, Sharma.; Manu, Singh. In vitro radical scavenging activity and phytochemical screening for evaluation of the antioxidant potential of *Operculina turpethum* root extract. J. Pharmacy Res., 2012; 5(2): 783-787.

[102] Edeoga, H.O., D.E. Okwu, B.; O. Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnology*, 2005, *4*, 685–688.

[103] Harborne, J. B.; Phytochemical Methods: A. Guide to *Modern Techniques of Plant Analysis*. 3rd Edn., Chapman and Hall, London, **1998**, *302*, 129-138.

[104] Fredua-Agyeman, M. The application of isothermal microcalorimetry for studying mixed probiotic cultures. Thesis, University College London, 2015.

[105] McChesney, J.D.; A.M. Clark, E.R. Silveira. Antimicrobial Diterpenes of Croton sonderianus, 1, Hardwickic and 3, 4-secotrachylobanoic acids. *J. of Natural Products*, **1991**, 54, 1625 – 1633.

[106] Arjun, P., S. Jha, P.N. Murthy, Manik, A. Sharone. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of*Hygrophila spinosa* T. Anders.*Int. J. Pharma. Sci. Res*. **2010**, *1*, 95-100

[107] Habib, M. R., Nikkon, F., Rahman, M. E.; Karim, M. R. Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantean* (Linn). Pakistan J. Bio. Sci. 2007, 10, 4174-4176.

[108] Patra, A., Jha, S., Murthy, P. N.; Manik, S. A. Isolation and characterization of stigmast-5-en-3*β*-ol (*β*-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int. J. Pharma. Sci. and Res.**2010**, 1, 95-100.

[109] Agrawal, P, Jain D, Gupta R, Thakur R. ^{13}C NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry. 1985, 24, 2479-2496.

[110] Kamboj, A.; Saluja, A. K. Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (*Asteraceae).Int. J. Pharma. Sci*. **2011**, *3*, 94-96.

[111] Arjun, P.; S. Jha, P.N. Murthy, Manik, A. Sharone. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of*Hygrophila spinosa* T. Anders.*Int. J.Pharma. Sci. and Res.***2010**, 1,95-100

[112] Venkata, S. P., Chaturvedula, Indra P. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus.* Chaturvedula and Prakash, *Int. Current Pharma. J*., 2012, *1*, 239-242.

[113] Hartmann, M. A. Plant sterols and membrane environment. *Trends in Plant Sci.*, **1998**, *3*, 170-175,

[114] Consolacion, Y.; Ragasa, Kathleen, L. Sterols from *Cucurbita maxima,Philippine J. Sci.* **2005**, *134*, 83-87

[115] Iftekhar, A.; Ridwan, I.; Amin, S.; Mohammad R., H.; Abdullah, A.; Mohammad A. Alkaloid, Sterol and Triterpenoids from *Glycosmis pentaphylla* (Retz.) DC. 2014, 13, 115-118,

[116] Dinan, L.; Whiting, P.;Sarkar, S. D.; Kasai, R.; Yamasaki, K. Assessment of natural products in the *Drosophila melangaster*cell bioassay for ecdysteroid agonist and antagonist activities. Cell Mol Life Sci. 2001, 58, 321-42.

7. APPENDIX

7.1 Spectroscopic data of MA 1 and Asc 1

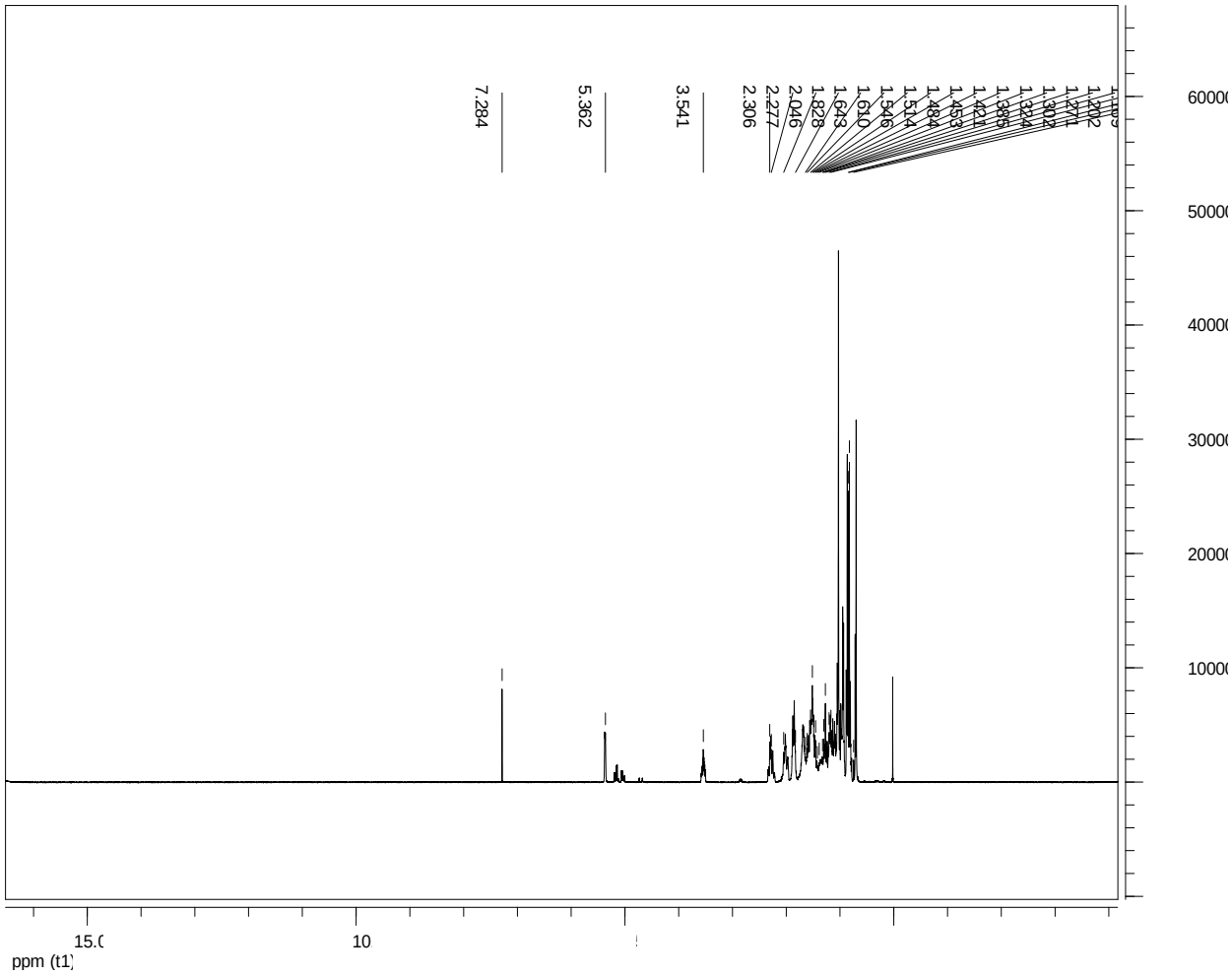


Figure 13: ^{1}H NMR spectrum of MA 1in $CDCl_3$as solvent.

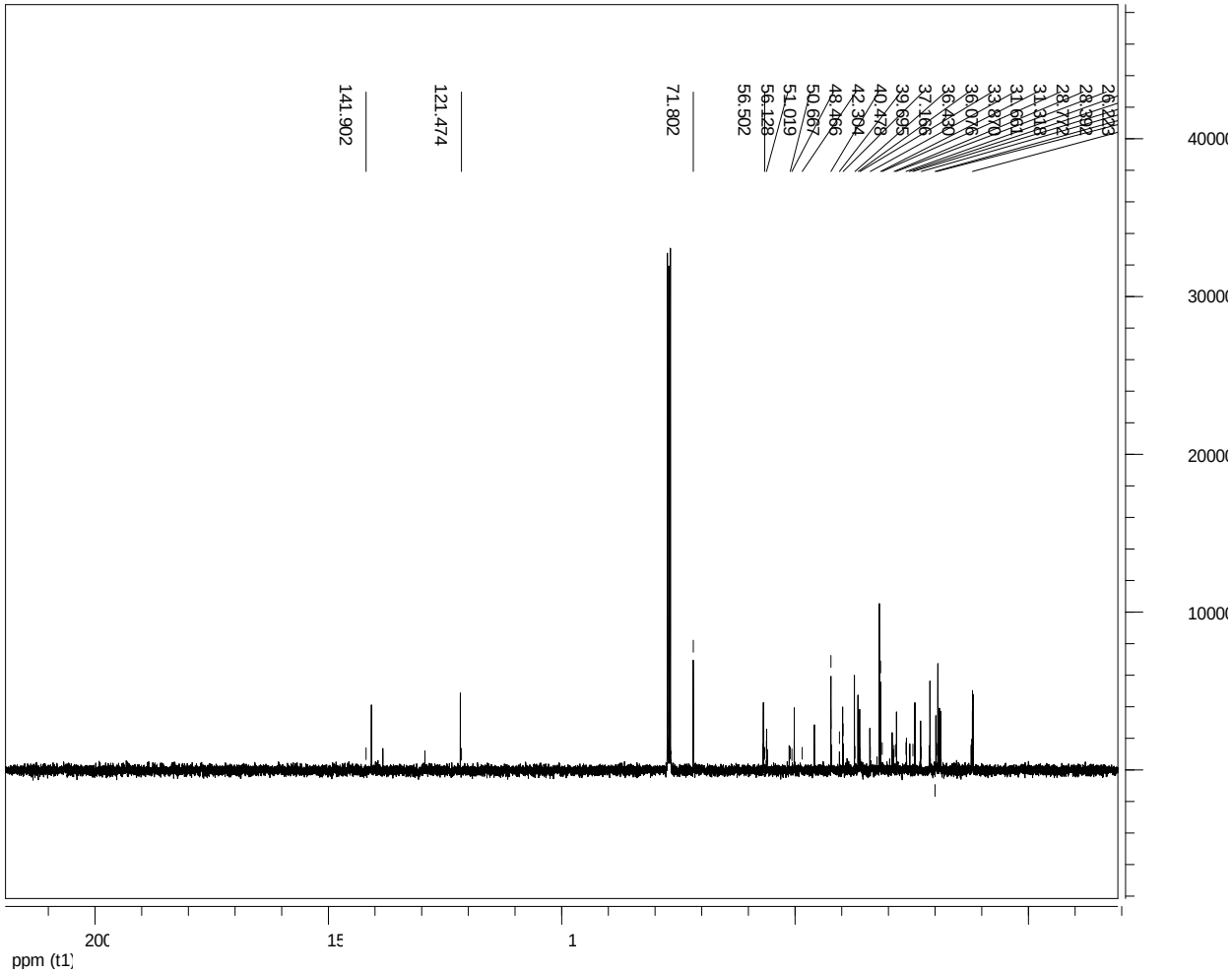


Figure 14:^{13}C NMR spectrum of MA 1in $CDCl_3$as solvent.

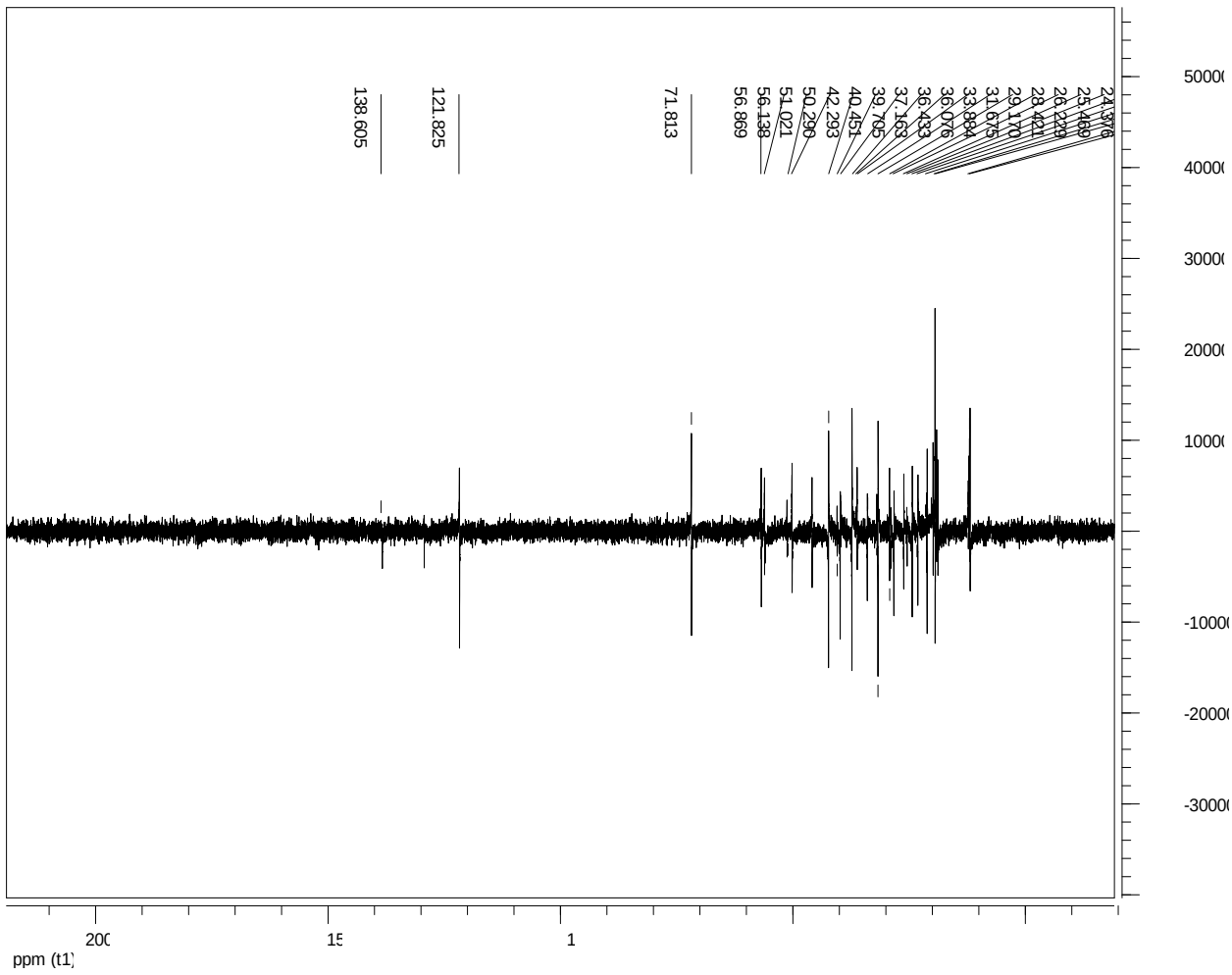


Figure 15:DEPT-135 spectrum of MA 1in $CDCl_3$as solvent.

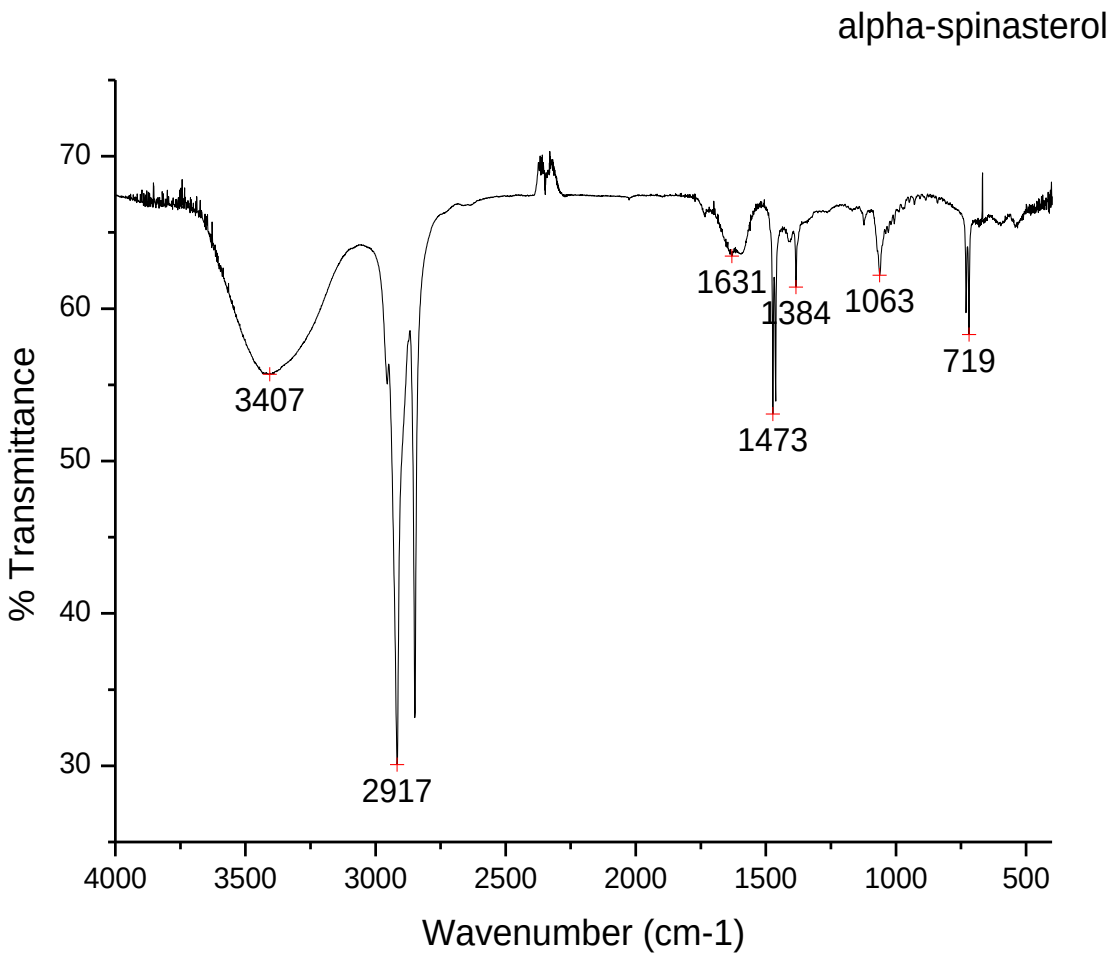


Figure 16:IR Spectrum of Asc 1

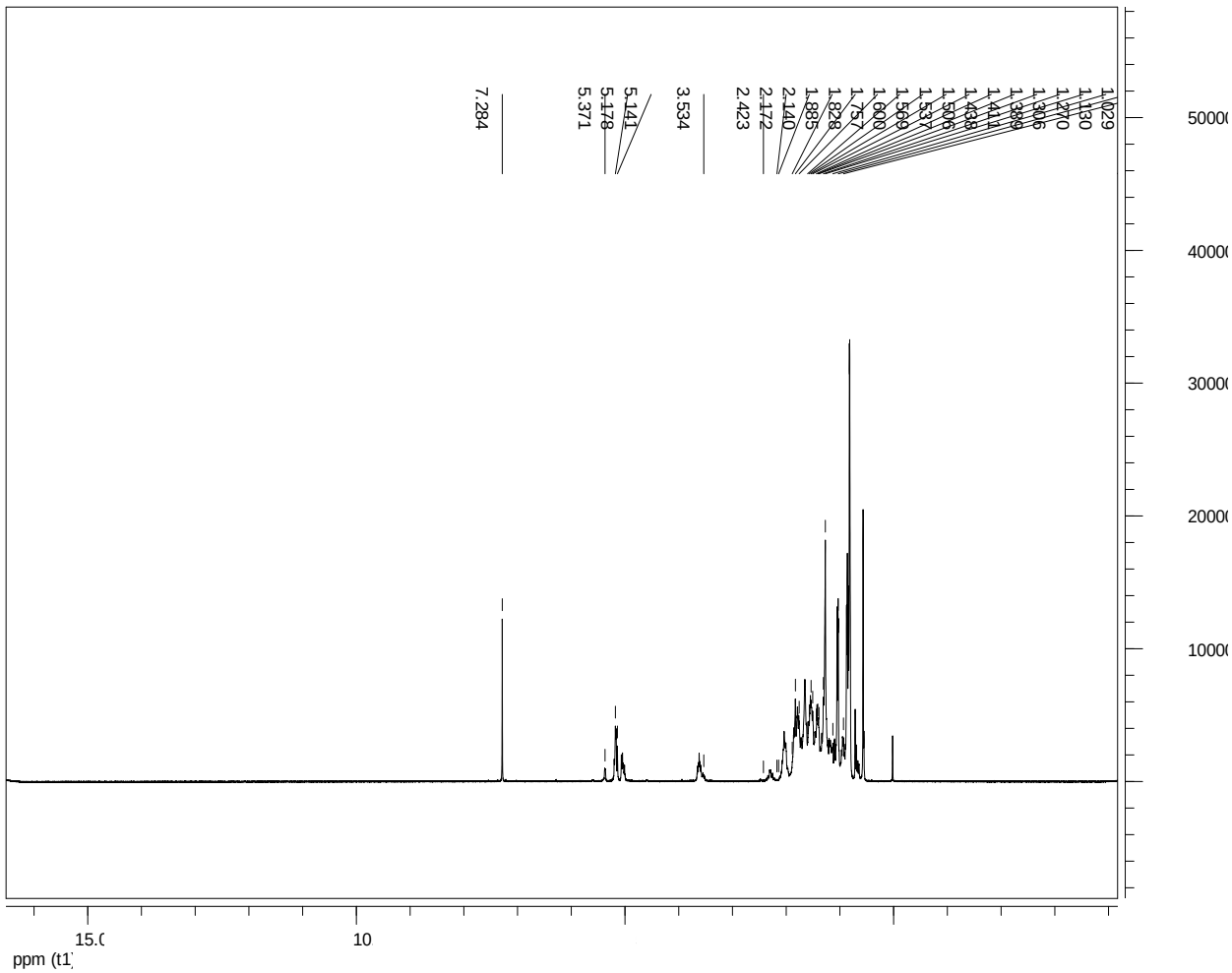


Figure 17: ^{1}H NMR spectrum of Asc 1in $CDCl_3$as solvent.

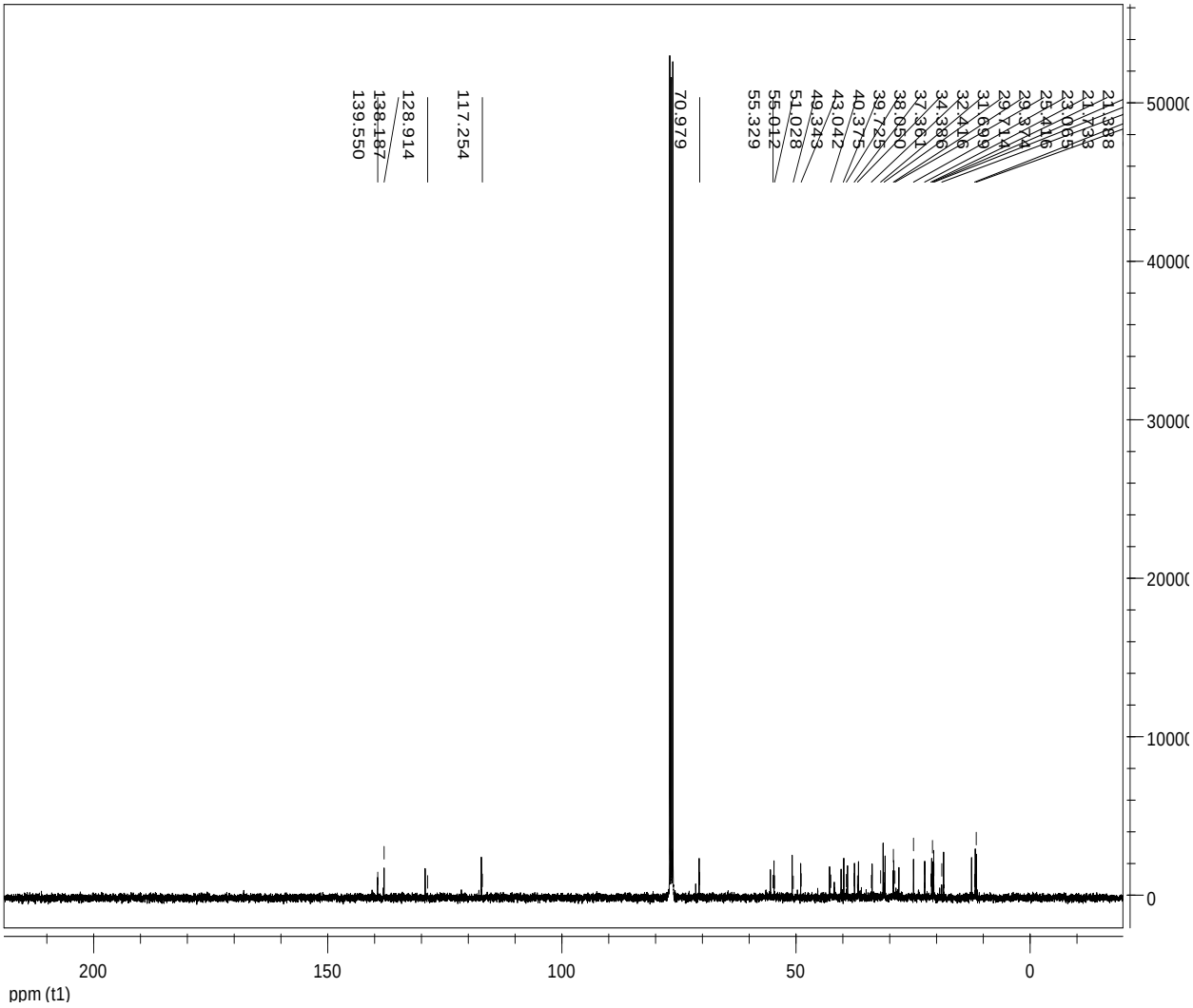


Figure 18: ^{13}CNMR spectrum of Asc 1in $CDCl_3$as solvent.

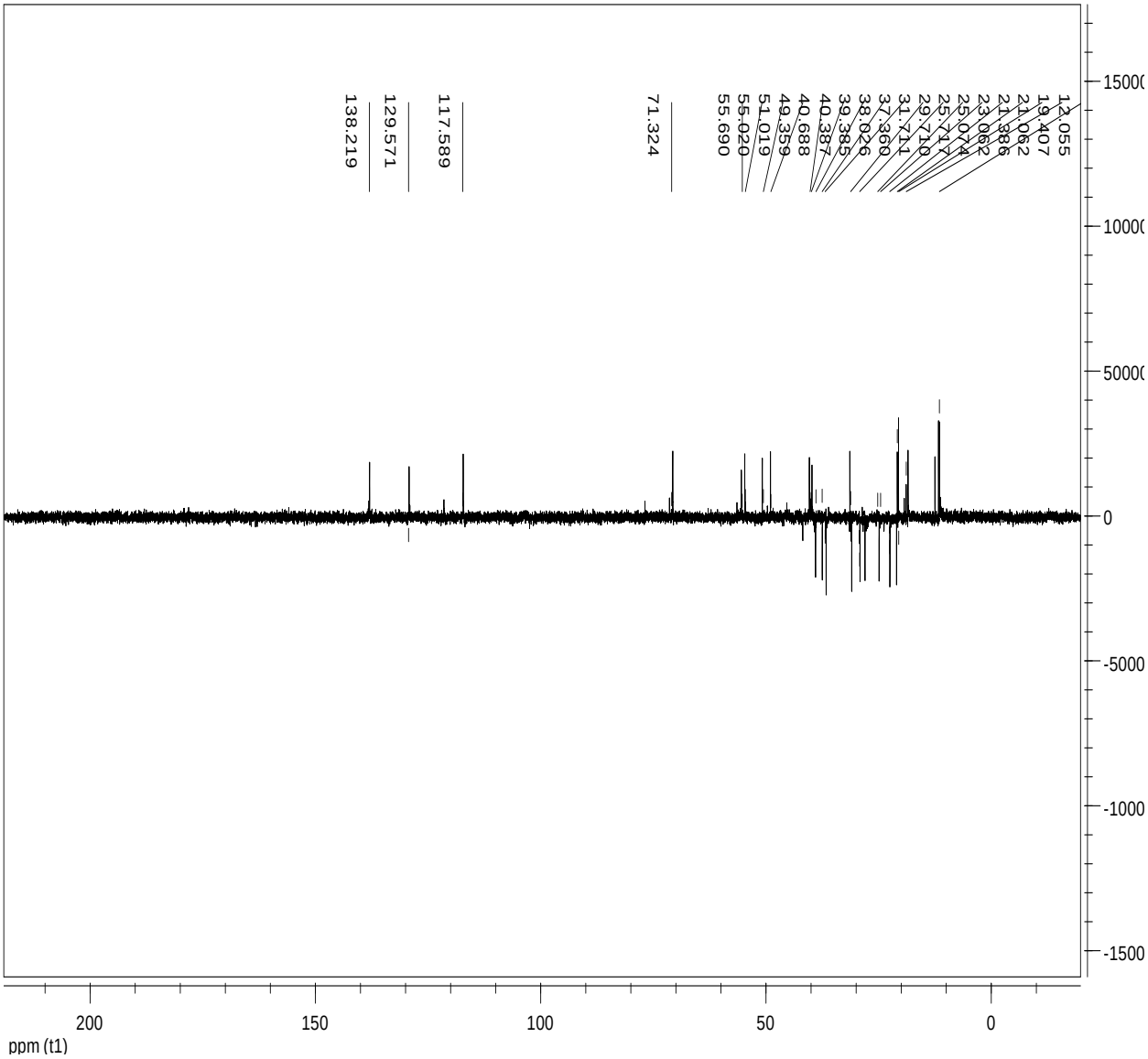
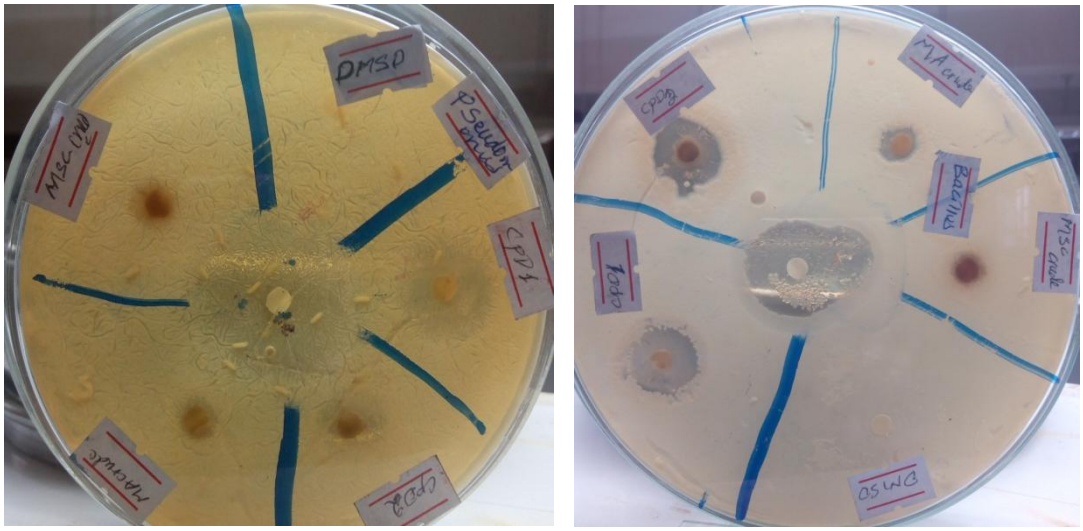


Figure 19:DEPT-135 spectrum ofAsc 1in $CDCl_3$as solvent.



Pseudomonas aeruginosaBacillussubtilis

Figure 20: Some of representative photograph during microbial activity taste

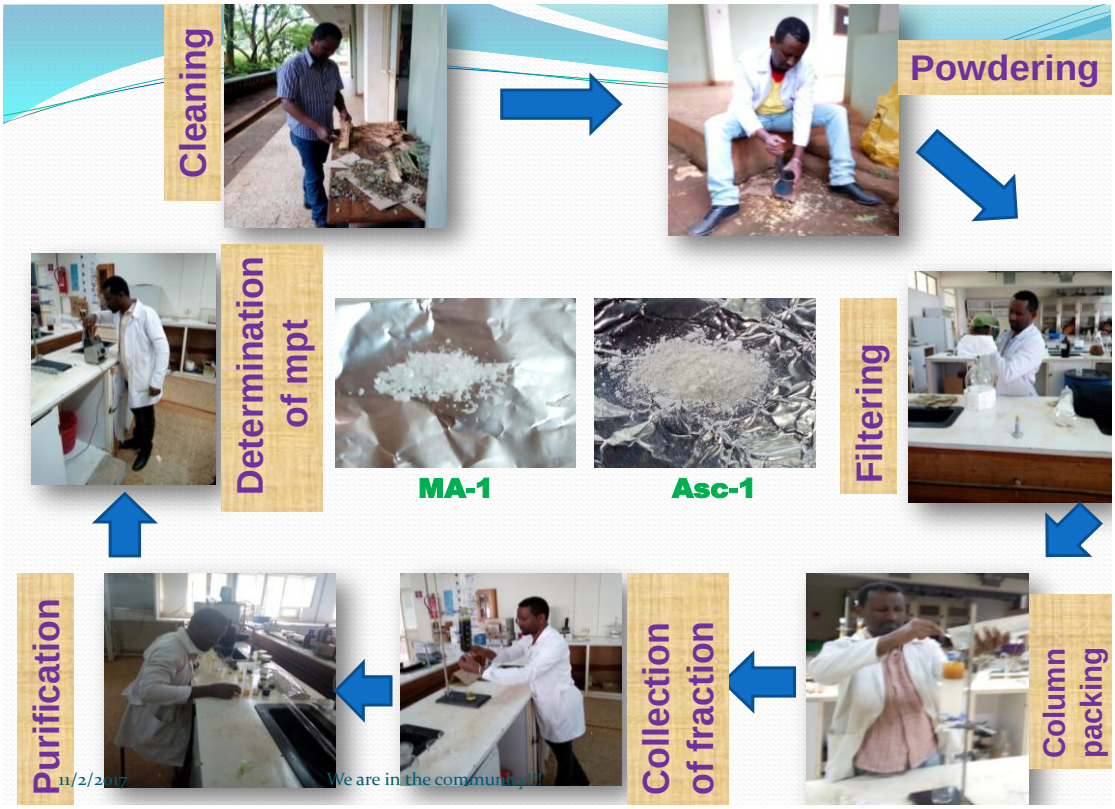


Figure 21: Photographs that show some of the practical work done throughout this study