Medicinal Chemistry

Fufa et al., Med Chem (Los Angeles) 2018, 8:6
DOI: 10.4172/2161-0444.1000507

Research Article Open Access

Isolation and Characterization of Compounds from the Leaves of *Melia azedarach* and Stem Bark of *Albizia schimperiana* and Evaluation for Antimicrobial Activities

Melkamu Feyera Fufa*, Fekadu Deressa, Tsegaye Deyou and Negera Abdisa

Department of Chemistry, College of Natural Science, Jimma University, Ethiopia

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 steroid whereas, 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* and stem bark of *Albizia schimperiana* afforded two pure compounds whose structure 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 (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and two fungi (*Aspergillus flavus* and *Fusarium* 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 of 50 mg/mL for the crude extracts, 20 mg/mL for the isolated compounds, it would be recommended for determination of MIC values 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

Introduction

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]. 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].

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 [11]. 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 [12]. 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 [13]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also

***Corresponding author:** Melkamu Feyera Fufa, Department of Chemistry, College of Natural Science, Jimma University, Ethiopia, Tel: +251925955783; E-mail: melkamufeyera@gmail.com

Received May 12, 2018; **Accepted** May 15, 2018; **Published** May 20, 2018

Citation: Fufa MF, Deressa F, Deyou T, Abdisa N (2018) Isolation and Characterization of Compounds from the Leaves of *Melia azedarach* and Stem Bark of *Albizia schimperiana* and Evaluation for Antimicrobial Activities. Med Chem (Los Angeles) 8: 154-165. doi: 10.4172/2161-0444.1000507

often with side effects. There is an urgent need to search new infection-fighting strategies to control microbial infections [14]. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin [15].

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 [16]. 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 [17]. *Melia azedarach* (Figure 1) (locally known as "Mimi") belongs to the family Meliaceae 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 [18,19]. Traditionally, different parts of *Melia azedarach* have been used by the local people of Mao-Komo special District in Benishangul Gumuz Regional State, Western Ethiopia, 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 (Figure 2) (locally known as 'Hambabeesa' in Afaan Oromo and 'Sesa' in Amharic) belongs to the family Fabaceae is an evergreen tree that grows at an altitude of 1600-2600 m [20]. Its 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 [21]. *Albizia schimperiana* is also locally used for the treatment of various bacterial infections (cough and diarrhea), antihelmintic activities, antimalarial activities, skin diseases. 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.

Materials and Methods

Chemicals used during this research work were products of Sigma-Aldrich of Analytical Reagent grade. The solvents used includes petroleum ether, ethanol, *n*-hexane, chloroform, ethyl acetate, methanol, sulphuric acid, sodium hydroxide, potassium hydroxide, hydrochloric acid, dimethyl sulfoxide, and Standard antibiotic drug (Gentamacin), antifungal drug (Mancozeb), Mueller Hinton agar, Petri dishes, plant extracts were used during antimicrobial test.

Melting point was measured using melting point apparatus (MFB 590 010T). NMR spectra, AVANCE II were processed 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 glass plates of 20 × 20 cm dimension, pre-coated with silica gel 60, F_{254} and silica gel was used for column chromatography (70-230 mesh size).

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, Ginijio Guduru kebele in Oromia Regional State, South Western Ethiopia. Both plants were identified at the Herbarium of the Department of Biology, Addis Ababa University. The Voucher specimens [No. 316 and 341 (ETH) respectively] were deposited at Addis Ababa University Herbarium. 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.

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 [22]. The extracts obtained were then filtered and concentrated using Rotary Evaporator (Laborata-4000) at 6°C to get the dried crude extracts of 48 g and 34 g, respectively (Figure 3).

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 [23-28] in $CHCl_3$/CH_3OH (1:1) extracts.

Test for alkaloids

Wagner's test: A fraction of extract was treated with Wagner's test

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

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

Figure 2: Picture of *Albizia schimperiana* leaves, Stem bark and its whole parts taken from Ginigo Guduru Kebele by FD in August, 2017.

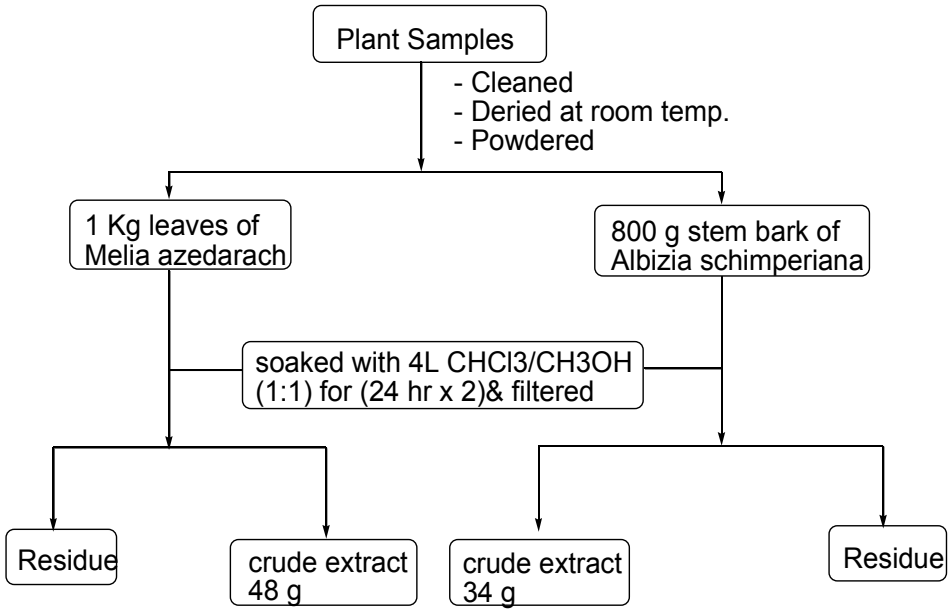

Figure 3: General procedures followed in the extraction of *Melia azedarach* and *Albizia schimperiana*.

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 [23].

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 [24].

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 [25].

Test for saponins: 2 ml 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 [26].

Test for terpenoids: To conduct this, 5 ml of plant extract is added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid. The presence of terpenoids gives a reddish brown color of interface [27].

Tests for steroid

Salkowski reaction: A few crystals of compounds (MA 1 and Asc 1) were dissolved in chloroform in different test tube and a few drops of concentrated sulphuric acid were added to the solution, both compounds (MA 1 and Asc 1) formed a reddish color in the upper chloroform layer [28] indicating presence of steroids.

Antimicrobial activity test

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

MA 1

Asc 1

Figure 4: The two characterized compounds (MA1and Asc1) from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana* respectively.

respectively. The standard drug for antibacterial taste (Gentamacin 10 mg/ml) and antifungal taste (Mancozeb 10 mg/ml). The culture media was prepared by dissolving 6.08 g of Muller Hinton Agar in 160 mL of distilled water and boiled to dissolve the media completely.

Preparation microbial cultures: The activity of the plant extracts were tested against four bacterial strains and two fungus which are disease causing infectious in living organism, two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus)* and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli)* and two fungus i.e., *Aspergillus flavus* and *Fusarium spp.* were used to evaluate antimicrobial activities. These standard bacterial strains were obtained from EPHI and preserved until used in the Department of Biology, Jimma University whereas; the fungi were obtained from the Department of Biology, Jimma University.

Antimicrobial assay

Antibacterial assay: The antibacterial activity test was done using disc diffusion method standard procedures [29] to test the extracts and isolated compounds against the bacteria strains. Muller Hinton Agar culture 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°C for 2 hours, then poured into sterile Petri dishes under sterile conditions. After the culture media was solidified, then 1 mL 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°C for 24 hours. After overnight incubation, the diameter of inhibitory zone formed around each discs were measured using ruler in mm and the observed results was recorded.

Antifungal activities test: A disc diffusion method was applied to test the plants extracts against the tested fungus [30] using standard antifungal agent Mancozeb as a positive control. The prepared culture media was autoclaved for 2 hours at 121°C temperature. After the culture media was solidified, then 1 mL of the fungal solutions was 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°C 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.

Results and Discussion

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 34 g 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 ether and ethyl acetate with increasing polarities.

Compounds isolated from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana*

Both columns were 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 and 185 fractions (50 ml each). The collected fractions were concentrated to dryness using a rotary evaporator at 65°C and were subjected to TLC analyses.

The chemical study of the leaves extract of *Melia azedarach* and stem bark of *Albizia schimperiana* afforded two pure compounds (Figure 4) whose structures were established as β-sitosterol and α-spinasterol respectively by extensive spectroscopic studies and direct comparison of their spectrum with published data.

Some of the physical properties of the two characterized compounds are mentioned as follows:

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

Preliminary 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 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,

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

NB: + sign indicates the presence of phytochemical constituents.
NB: - sign indicates the absence of phytochemical constituents.

Table 1: Bioactive components of *Melia azedarach* leaves extract and *Albizia schimperiana* stem bark extract ($CHCl_3/CH_3OH$).

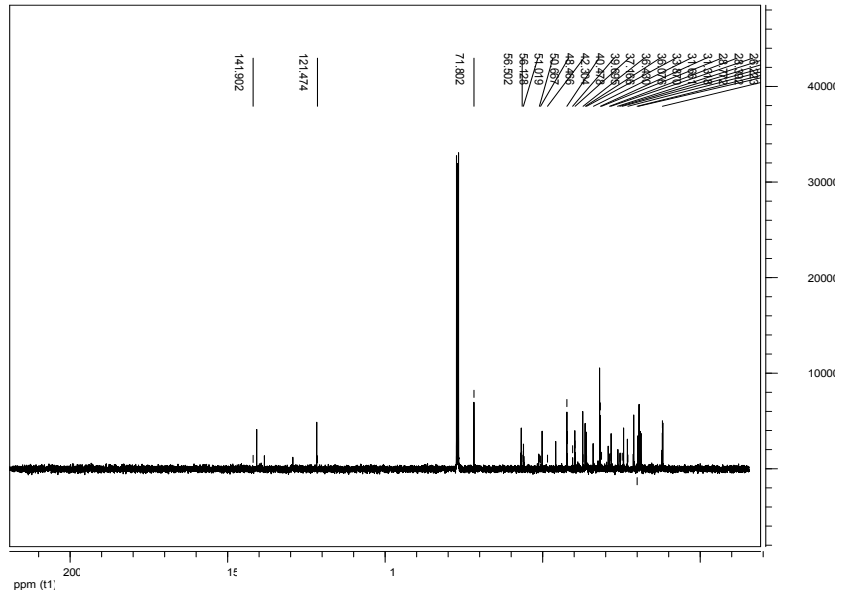

Figure 5: ^{1}H NMR spectrum of MA 1 in $CDCl_3$ as solvent.

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).

Structural elucidation of the isolated compounds

Structural elucidation of MA 1 from leaves extract of Melia azedarach: The ^{1}H NMR spectroscopic data of MA 1, (Figure 5) 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 and one olefin proton at δ 5.34 ppm.

The ^{13}C NMR spectroscopic data of **MA 1**, (Figure 6) 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 ppm were assigned for the olefin carbons C-5 and C-6 and 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. From DEPT-135 (Figure 7), 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 [31,32]. Based on the spectroscopic data and comparison with literature, **MA 1** was identified as β-sitosterol with

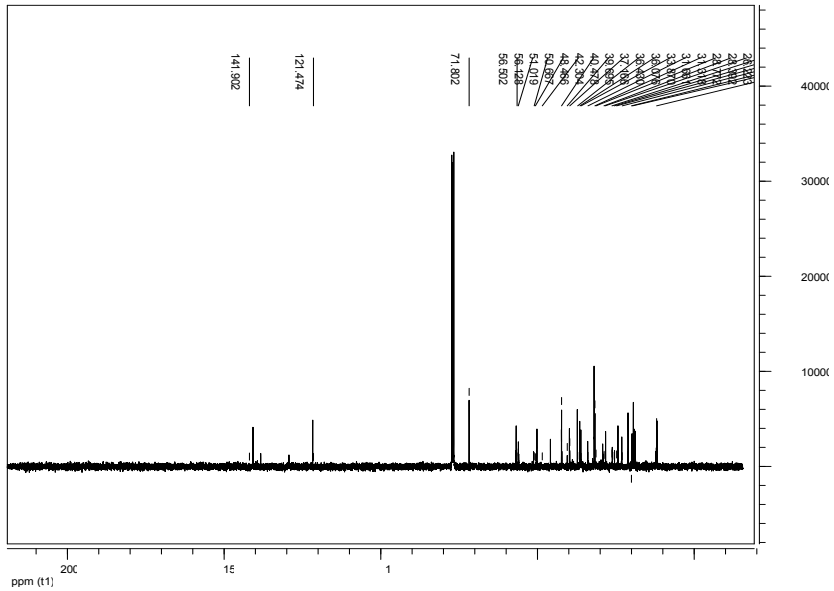

Figure 6: ^{13}C NMR spectrum of MA 1 in $CDCl_3$ as solvent.

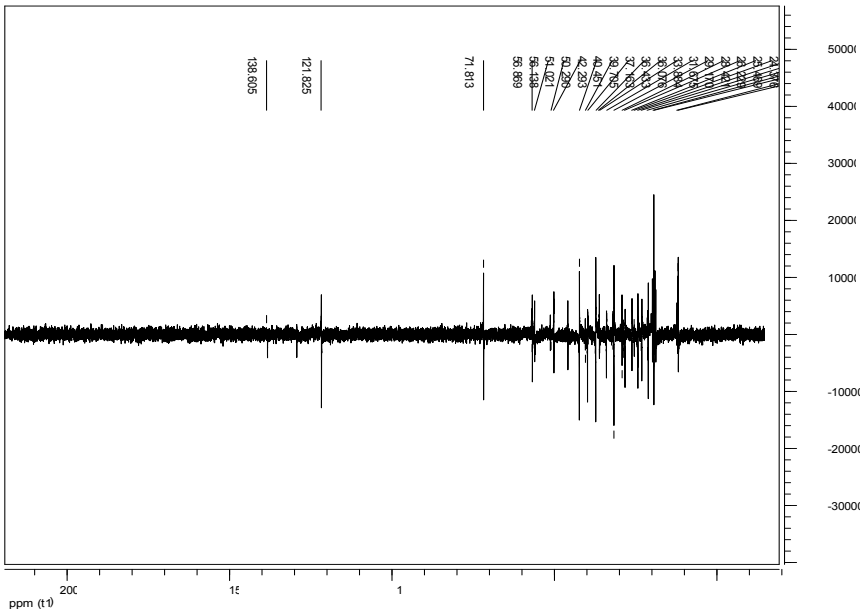

Figure 7: DEPT-135 spectrum of MA 1 in $CDCl_3$ as solvent.

a molecular formula of $C_{29}H_{50}O$ (Figure 8). The compound further confirmed by comparing its NMR spectrum data with those reported in previous studies (Table 2) [33,34].

Structural elucidation of Asc 1 from stem bark extract of Albizia schimperiana: Asc 1 isolated as colorless crystalline solid with melting point 134°C-136°C. On subjection to IR spectroscopic analysis (Figure 9), 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 719 cm^{-1}. The ^1H NMR spectroscopic data of Asc 1, (Figure 10) 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 multiplet at δ 3.52 ppm. Two olefin protons appeared at δ 5.17 (1H, dd) and 5.16 (1H, dd) in the ^1H NMR spectrum for H-22 and H-23, respectively.

The ^{13}C NMR spectroscopic data of **Asc 1**, (Figure 11) gave signal

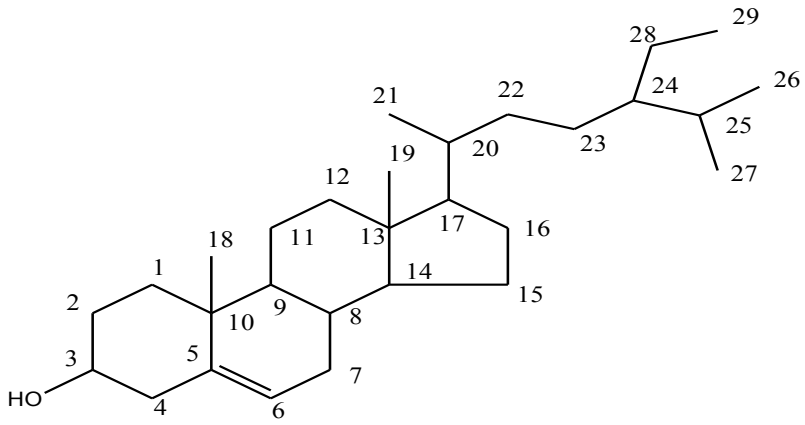

Figure 8: Structure of β-stiosterol from the leaves extract of *Melia azedarach.*

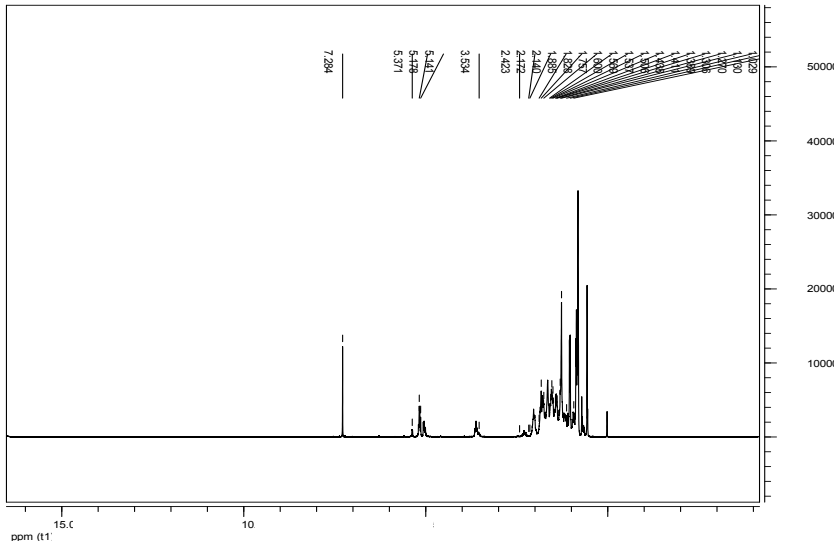

Figure 9: ^{1}H NMR spectrum of Asc 1 in $CDCl_3$ as solvent.

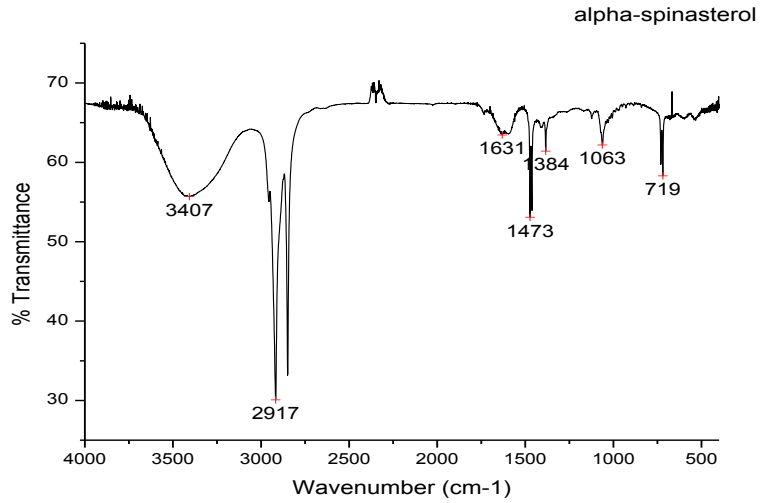

Figure 10: IR Spectrum of Asc 1.

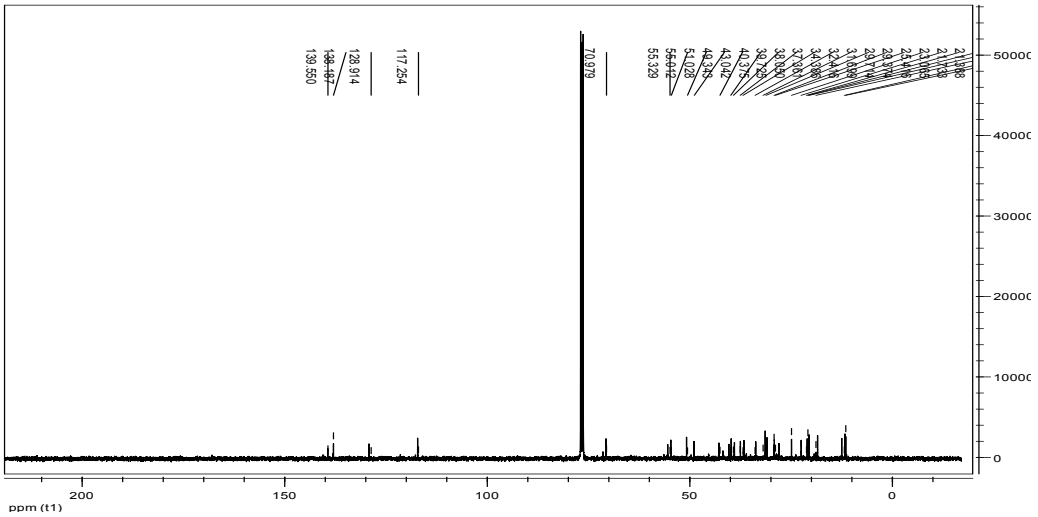

Figure 11: ^{13}CNMR spectrum of Asc 1 in $CDCl_3$ as solvent.

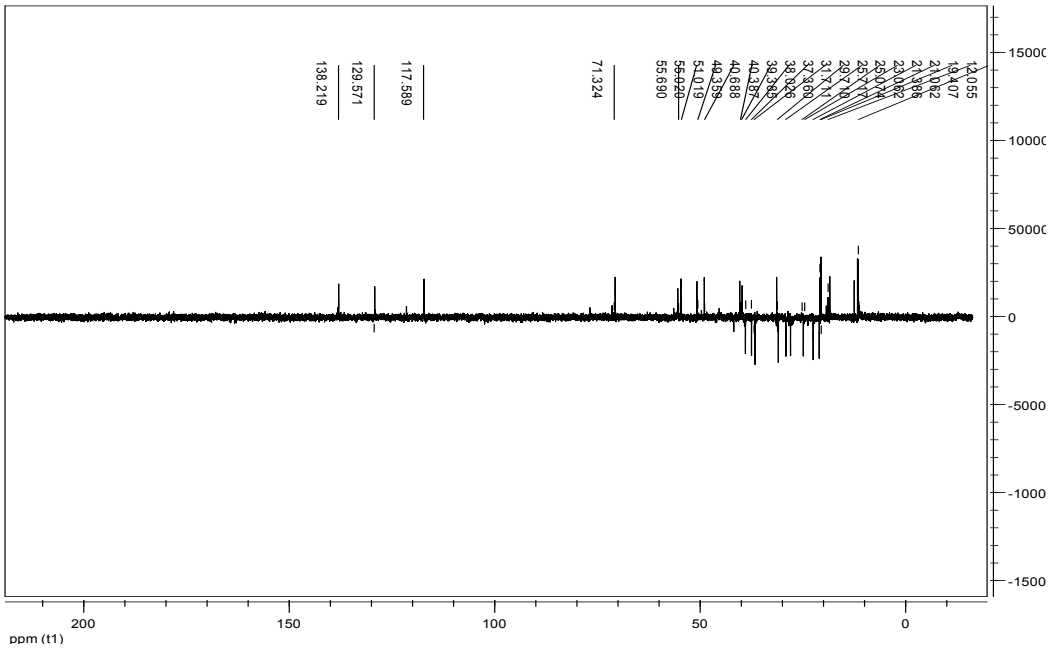

Figure 12: DEPT-135 spectrum of Asc 1 in $CDCl_3$ as solvent.

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 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. 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 12) 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 data and the other physical properties, the isolated compound was in good agreement for the structure of α-spinasterol having a molecular formula of $C_{29}H_{48}O$ [37]. Therefore **Asc 1** was identified as α-spinasterol (Figure 13). The NMR spectrum of this compound resembled to the data published in previous studies (Table 3) [38]. This is the first report of isolation of α-spinasterol from the species of *Albizia schimperiana* but not for the genus *Albizia*.

Antimicrobial activity data: In the present investigation, the crude extracts and isolated compounds of both plants were 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 were recorded. The effectiveness

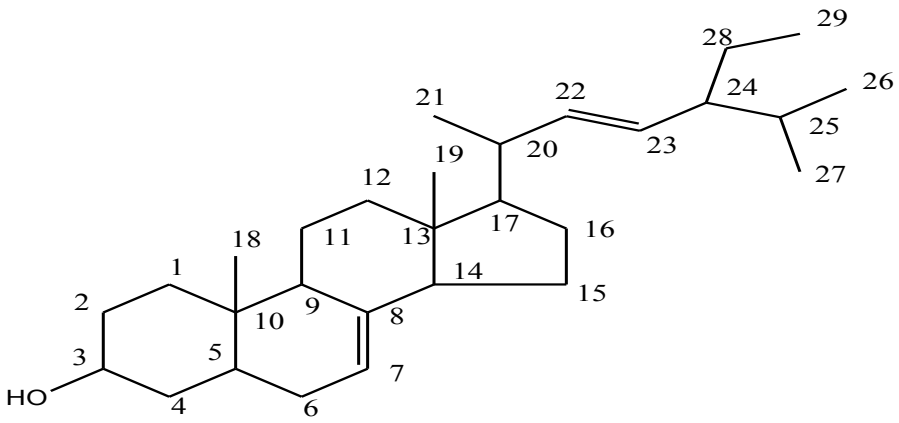

Figure 13: Structure of *α*-spinasterol from the stem bark of *Albizia schimperiana*.

Carbon atom	^{1}H NMR Experimental	^{1}H NMR Literature	^{13}C NMR Experimental	^{13}C NMR 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

Table 2: Chemical shifts of ^{1}H NMR and ^{13}CNMR of MA 1 with the reported data in literature [35,36].

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

Table 3: Chemical shifts of ^{1}H NMR and ^{13}C NMR of Asc 1 with the reported data in literature [37].

Test sample	Concentration 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
NB+: Gram positive bacteria
MA 1: β-stiosterol
Asc 1: α-spinasterol
NI: Not inhibited
MA: *Melia azedarach*
Asc: *Albizia schimperiana*

Table 4: Antibacterial activity of the test samples with the standard drug solution (Gentamacin).

Test sample	Concentration mg/mL	Diameter of zone inhibition of each tested organism (mm)	
		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
NB+: Gram positive bacteria
MA 1: β-stiosterol
Asc 1: α-spinasterol
NI: Not inhibited
MA: *Melia azedarach*
Asc: *Albizia schimperiana*

Table 5: Antifungal activity of the test samples with the standard drug solution (Mancozeb).

of the samples was also examined among each other against the tested pathogens by comparing the maximum zone of inhibition.

Antibacterial activity test results data: 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 schimperiana* 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 4.

MA 1 (β-stiosterol) has shown more effective antibacterial activities than that of compound 2a with the comparison of the standard drug (Gentamacin) as demonstrated by the observed inhibition zone values (Table 4). The obtained results also indicated that **MA 1** (β-stiosterol) exhibit relatively higher zone of inhibition against two bacterial strains namely, *Bacillus subtilis* (19.9 mm) and *Pseudomonas aeruginosa* (22.8 mm) with the comparison of standard drug Gentamacin (28 mm and 40 mm) respectively (Supplementary Figure 1). 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*. 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*.

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 fungus were assessed quantitatively on the basis of inhibition zone and shown in Table 5.

The observed results showed that all samples were active against the tested fungus (*Aspergillus flavus* and *Fusarium* spp.) except the crude extract of *Albizia schimperiana* 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 5).

Conclusion

Medicinal plants are rich sources of a wide variety of chemical compounds and have been used as a major constituent 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. The present study resulted 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 schimperiana* identified as α-spinastestrol. In the study, the isolated compounds as well as the crude extract of both plants were 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. On comparing plants, the crude extract as well as the isolated compound of *Melia azedarach* inhibited the growth of the tested four bacterial strains and two fungal more effectively. The overall observation indicated that the leaves extract of *Melia azedarach* has relatively active medicinal values than that of the stem bark of *Albizia schimperiana*. The scientific findings of the present study support the folklore claim along with the therapeutic application of both plants against microbial activities from *in vitro* assay result and an important step towards their acceptance and development of modern drugs.

Recommendations

- The present study simply focused on the evaluation of antimicrobial activities of the leaves of *Melia azedarach* and the stem bark of *Albizia schimperiana* medicinal activities (Supplementary Figure 2), it would be recommended for the determination of MIC values provides a quantitative measure for the level of resistance expressed by the test organism.

- 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.

Acknowledgements

We would like to acknowledge Department of Chemistry, College of Natural Science, Jimma University, for funding this project, Addis Ababa University for NMR data, Ethiopia Public Health Institute (EPHI) for providing bacterial strains for biological data.

References

1. Abdullahi AA (2011) Trends and challenges of traditional medicine in Africa. Afr J Trad Complem Alte Med 8: 115-123.
2. WHO (2000) General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland.
3. Bodeker G (1994) Traditional health knowledge and Public policy. Nature and Resource 30: 5-16.
4. Cardini F, Wade C, Regalia AL (2006) Clinical research in traditional medicine: priorities and methods. Complem Alt Med 14: 282-287.
5. Davidson-Hunt I (2000) Ecological ethno botany: stumbling toward new practices and paradigms. MASA J 16: 1-3.
6. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY (2011) Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. Afr J Traditional Complem Alter Med 8: 1-10.
7. World Health Organization (1993) Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila: WHO Regional Office for the Western Pacific.
8. UNESCO (1996) Culture and Health, Orientation Texts World Decade for Cultural Development, p: 129.
9. Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, et al. (2005) Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. J Ethnopharmacol 97: 421-427.
10. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY (2011) Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. Afr J Trad Complem Alter Med 8: 1-10.
11. Cowan MM (1999) Plant products as antimicrobial agents. Clinical Microbiology Reviews 12: 564-582.
12. Singh SB, Barrett JF (2006) Empirical antibacterial drug discovery-foundation in natural products. Biochemical Pharmaco 71: 1006-1015.
13. AlBari MA, Sayeed MA, Rahman MS (2006) Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomyces bangladeshiensis. A novel species collected in Bangladesh. Res J Med Sci 1: 77-81.
14. Sieradzki K, Wu SW, Tomasz A (1999) Inactivation of the methicillin resistance gene mecA in vancomycin-resistant Staphylococcus aureus. Micro Drug Resist 5: 253-257.

15. Kameshwara K, Rao C (2000) Database of medicinal plants: Government of Karnataka, India. pp: 1-23.

16. Byarugaba DK (2004) A view on antimicrobial resistance in developing countries and responsible risk factors. Int J Antimicrobial Agents 24: 105-110.

17. Abreu AC, Borges A, Simões LC, Saavedra MJ, Simões M (2013) Antibacterial activity of phenyl isothiocyanate on Escherichia coli and Staphylococcus aureus. Med Chem 9: 756-761.

18. Suresh K, Deepa P, Harisaranraj R, Vaira AV (2008) Antimicrobial and Phytochemical investigation of the leaves of Carica papaya, Cynodondactylon, Euphorbia, Hirta, Melia azedarach and Psidiumguajava. Ethnobotanical Leaflets 12: 1184-1191.

19. Nahak G, Sahu RK (2010) In vitro antioxidative activity of Azadirachta indica and Melia azedarach leaves by DPPH scavenging assay. J Am Sci 6: 123-128.

20. Tewelde N, Abebe G, Eisler M, McDermott J, Greiner, M (2004) Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia. Acta Trop 90: 163-170.

21. King A, Young G (1993) J American Dietetic Association 24: 213.

22. Chan C, Ngoh G, Yusof R (2012) A brief review on anti-diabetic plants: global distribution, active ingredients, extraction techniques and acting mechanisms. Pharma Review 6: 22-28.

23. Lalitha TP, Jayanthi P (2012) Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. Asian J Plant Sci Res 2: 115-122.

24. Onwukaeme DN, Ikuegbvweha TB, Asonye CC (2007) Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of Pycanthus angolensis Weld Warb (Myristicaceae) on corneal ulcers in rabbits. Tropical J Pharma Res 6: 725-730.

25. Kikuzaki H, Nakatani N (1993) Antioxidant effects of some ginger constituents. J Food Sci 58: 1407-1410.

26. Sharma V, Singh M (2012) In vitro radical scavenging activity and phytochemical screening for evaluation of the antioxidant potential of Operculina turpethum root extract. J Pharmacy Res 5: 783-787.

27. Edeoga HO, Okwu DEB, Mbaebie O (2005) Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnology 4: 685-688.

28. Harborne JB (1998) Phytochemical Methods: A. Guide to Modern Techniques of Plant Analysis. 3rd edn. Chapman and Hall, London 302: 129-138.

29. Fredua-Agyeman M (2015) The application of isothermal microcalorimetry for studying mixed probiotic cultures. Thesis, University College, London.

30. McChesney JD, Clark AM, Silveira ER (1991) Antimicrobial Diterpenes of Croton sonderianus, 1, Hardwickic and 3, 4-secotrachylobanoic acids. J Natural Products 54: 1625-1633.

31. Habib MR, Nikkon F, Rahman ME, Karim MR (2007) Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of Calotropis gigantean (Linn). Pakistan J Bio Sci 10: 4174-4176.

32. Patra A, Jha S, Murthy PN, Manik SA (2010) Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of Hygrophila spinosa T Anders. Int J Pharma Sci Res 1: 95-100.

33. Agrawal P, Jain D, Gupta R, Thakur R (1985) ^{13}C NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry 24: 2479-2496.

34. Kamboj A, Saluja AK (2011) Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae). Int J Pharma Sci 3: 94-96.

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

36. Venkata SP, Chaturvedula IP (2012) Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of Rubus suavissimus Chaturvedula and Prakash. Int Current Pharma J 1: 239-242.

37. Consolacion Y, Ragasa KL (2005) Sterols from Cucurbita maxima. Philippine J Sci 134: 83-87.

38. Iftekhar A, Ridwan I, Amin S, Mohammad RH, Abdullah A, et al. (2014) Alkaloid, Sterol and Triterpenoids from Glycosmis pentaphylla (Retz.) DC. BJOL 13: 115-118.