

Evaluation of Antibacterial Activities of Compounds Isolated From *Sida rhombifolia* Linn. (*Malvaceae*)

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Abstract

The main objective of this study was to isolate compounds from roots of *Sida rhombifolia* and subsequently evaluate their antibacterial activities. Crude gradient extracts were obtained from three solvents (petroleum ether, chloroform and methanol) with increasing solvent polarity using cold maceration technique. The *in vitro* antibacterial activity evaluation of gradient extracts and isolated compounds was done on four different pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) using agar disc diffusion technique. The results showed that antibacterial activities were comparable to each other. But their activities were relatively weaker as compared to that of the reference compound (ciprofloxacin). Among the three crude extracts, the chloroform extract was subjected to column chromatographic separation that led to isolation of SRL-1, SRL-2 and SRL-3. The chemical structures of the compounds were found to be *n*-hexacos-11-enoic acid, stigmasterol and β -sitosterol, respectively, based on physical properties and spectroscopic (IR and NMR) data as well as literature reports. The observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections. Thus, further test is recommended on large number of bacterial strains to decide the potentials of the compounds as candidates in development of antibacterial drugs.

Keywords: *Sida rhombifolia*; Extraction; Isolation; Antibacterial activity; *n*-Hexacos-11-enoic acid; Stigmasterol; β -Sitosterol

Introduction

Use of natural products for curing wide variety of human and domestic animal diseases has a long history that goes to human civilization. These products have been used as good sources of many modern drugs for treatment of several human diseases such as cardiovascular, cancer, malaria, mental diseases, etc. Most of these modern drugs have been obtained or discovered from medicinal plants [1-7]. Such drugs have been discovered after observing the medicinal use of a particular plant or its parts (leaves, roots, barks, fruits or seed or whole plant) by herbalists, and subsequent isolation of bioactive compounds from the plant or part of the plant that was traditionally used for treatment of different human illnesses [8,9]. All these facts indicate that medicinal plants still have an immense potential as sources of modern drugs.

Sida rhombifolia is one of the 200 species in *Sida*. It grows in tropical and warm regions, and distributed throughout the tropics [10,11]. *Sida rhombifolia* Linn is known for its wide range of medicinal uses. For instance, it is used for treating stings and bites of scorpion, snake and wasp (its flowers), skin diseases and sores (its stem), treat stomach disorders, stomach pain, digestion problem (its roots), malaria, flatulence, diarrhea (its root decocted), dysentery (roots), irritable bowel syndrome, gastritis, enteritis, hemorrhoids (its roots and leaves), diabetes (its leaves), chicken pox, blood cleaning, fatigue [11,12], headache and migraine headache (its fruits), eye problems, conjunctivitis, toothaches (roots), fever, gum infection, swelling, tonic, wounds (root and leaves) [13], ophthalmia and swelling (its leaves), cuts and wounds (its leaves) are some of the example to mention [14-16]. In Ethiopia, *Sida rhombifolia* is also widely used by herbalists to treat different human diseases. These include use of its leaves to treat skin disease, wounds and inflammations [17], rabies and skin bleeding [18]. In Jimma area, it is locally known as *karaba*, and its stems are used as tooth brush and the leaves and stem barks are used for the treatment of wound [19].

There are reports on scientific studies on evaluation of biological

activities of extracts from different morphological parts of *Sida rhombifolia*. *In vitro* antibacterial activity test of aqueous-methanol extract of the whole part of *Sida rhombifolia* showed effective antibacterial activities [11]. Methanolic extract of its fruit was found to show significant *in vitro* antibacterial activities against several bacteria species [20]. Reports also indicated the ability of leaf extract to ameliorate GM-induced nephrotoxicity and renal dysfunction as demonstrated from the results of animal model study [21]. In another report, it has been discussed that methanolic extract of its aerial part showed anti-inflammatory activity in animal model study [22]. Ethanol and aqueous extracts of aerial parts of the plant were also reported to be useful in the treatment of arthritis [23]. A recent report from experimental result of Ranjan et al. [24] also showed significant anti diarrheal activity of methanolic extract of *Sida rhombifolia*. An *in vitro* study using ethanol extract of roots, stems, leaves, and whole plant showed antioxidant activities. This indicated that *Sida rhombifolia* could be promising a source of natural antioxidants [25,26]. Similar results were also reported recently using root and stem extracts on animal models [27]. Aqueous extract of leaves was administered to hyperbilirubinemic rats, and showed potential of this plant as source new drugs for hyperbilirubinemic subjects [28]. A study carried out, in Bangladesh, indicated that ethyl acetate extract of its leaves showed potent cytotoxicity that was comparable to reference standard, gallic acid, and weak antibacterial activity against both Gram-positive and Gram-negative test organisms [29]. Recent reports, on the other hand, indicated that aqueous-methanol extract of *Sida rhombifolia* showed

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significant antibacterial activity (against pathogenic bacteria involved in diarrhea) [16]. Antidiabetic properties of aqueous extract leaves of *Sida rhombifolia* using normal and streptozotocin-induced diabetic rats was also reported recently. The extract showed good hypoglycemic and hypolipidemic effects. The results were claimed to provide scientific evidence in favor of the traditional use of *Sida rhombifolia* leaves for the treatment of diabetes mellitus [30]. A report by Poojari et al. indicated chemo preventive and hepatoprotective potentials of seed extract as demonstrated by its effect in rats of diethylnitrosamine-hepatocellular preneoplastic foci and carbon tetrachloride-induced hepatotoxicity [31]. Ethyl acetate and aqueous extracts of *Sida rhombifolia* was also reported to show marked antibacterial activity (against *K. pneumonia*, *S. aureus*, and *S. mutans*) and significant antifungal activity (against *A. niger*, *C. albicans* and *M. gypseum*) [32].

The results obtained from biological activity tests of crude extracts of *Sida rhombifolia* have initiated researchers to carry out isolation and characterization of compounds from the plant (or its parts) which subsequently subjected to biological activity tests in order to evaluate their potential as leads in the drug discovery processes for treating human diseases. Phytochemical analyses the fruits, roots, leaves and stem of *Sida rhombifolia* revealed the presence of saponins, tannins, amino acids, fatty acids, sterolic compounds, alkaloids, terpenoids, carbohydrates, lignans, glycosides, phenolics, steroids and flavonoids. Notably, both tannins and phenolics have been reported to possess antibacterial activities [27,33,34].

The presence of ecdysteroids and/or their glycosides in *Sida rhombifolia* was reported by Yang-Hong et al. [35] and Jadhav et al. [36]. Isolation of this group compounds was reported from methanol extract of the whole plant parts of the plant. The ecdysteroids were 20-hydroxyecdysone-3- β -D-glucopyranoside, 20-Hydroxyecdysone, pterosterone-3- β -D-glucopyranoside, ecdysone, ecdysone-3- β -D-glucopyranoside, ecdysone and 20-hydroxy- (25-acetyl) ecdysone-3- β -D-glucopyranoside [37]. The detection or characterization of 20-Hydroxyecdysone from *Sida rhombifolia* using HPLC method was previously reported by Jadhav et al. [38]. Soxhlet extraction of stem of *Sida rhombifolia* with methanol, followed by solvent-solvent partitioning using chloroform, petroleum ether and ethyl acetate gave single glycoside known as phenyl ethyl- β -D-glucopyranoside. The report indicated isolation of this compound from *Sida rhombifolia* for the first time. *In vitro* antibacterial activity test of the compound showed that it is effective against several bacteria strain [39]. The compound was also found to show larvicidal activity against common filarial vector [29]. Daucosterol was recently isolated from the *n*-hexane soluble fraction of methanolic extract of the stems of *S. rhomboidea* [40]. The authors also reported antimicrobial and antioxidant activities of *n*-hexane, carbon tetrachloride and dichloromethane soluble fractions of the methanol extract. The results indicated that the dichloromethane and carbon tetrachloride soluble fractions showed moderate inhibitory activity to microbial growth while the *n*-hexane fraction showed highest cytotoxicity. The dichloromethane soluble fraction also revealed potent antioxidant activity [40]. Isolation and characterization of alkaloid constituents such as β -phenethylamine, ephedrine, ψ -ephedrine, quinazoline such as vasicine, vasicinol, vasicinone, carboxylated tryptamines such as S- (+)- N_b -methyltryptophan methyl ester, choline and betaine from aerial *Sida rhombifolia* were reported by Prakash et al. [41]. Sterols (β -sitosterol, stigmasterol, campesterol, stigmasterol, spinasterol and cholesterol), *n*-alkanes (e.g., nonacosane and hentriacontane) and *n*-alcohols were also identified/reported from dried whole and aerial parts of *Sida rhombifolia* [37,42-44]. As discussed above, crude extracts of different part of *Sida rhombifolia* including its

roots showed effective antibacterial activities [11,24,45]. However, there are no reports on the evaluation of antibacterial activities of compounds isolated from its roots. Thus, in our study efforts were made to isolate and characterize compounds from the root parts of *Sida rhombifolia* Linn, and to evaluate their antibacterial activities.

Methods and Materials

Chemicals and apparatus

General laboratory grade solvents such as petroleum ether, chloroform, ethyl acetate, acetone and methanol (Purchased from supplied by Sigma Aldrich Chemicals Co. Ltd) and distilled water for extraction and column elution. Silica gel (60-120 mm mesh size) and TLC (silica gel, UV-254) pre-coated on aluminum sheets were used for chromatographic analyses. Compound spots on TLC plates were detected using UV (uvitec chamber) and iodine vapor. Evaporation of solvent was carried out using a rotary evaporator (Heidolph, UK) and HY-5A Manoeuvre style vibrator (Rotary shaker) were used for extraction. A standard antibiotic disc (ciprofloxacin, 5 μ g) and culture medium (Mueller Hinton agar, nutrient broth) were used for the antibacterial activity test. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and DEPT-135 were recorded using Bruker Advance 400 MHz spectrometer. CDCl_3 was used as a solvent in all spectroscopic analysis. Infrared (IR) spectra (KBr) were obtained from Perkin-Elmer BX infrared spectrometer (400-4000 cm^{-1}). Melting point apparatus (Griffin) was used for melting point determination. All spectroscopic analysis were carried out at the Department of chemistry, Addis Ababa University.

Collection of plant material and extraction

Sida rhombifolia Linn was collected in November 2011 from Sokoru District, Jimma Zone and South-western Ethiopia. The collected plant material was dried at room temperature without exposing it to direct sun light. The dried material was then milled using a mechanical grinder. Botanical identification was made by Dr Remesh (a botanist) and a specimen is deposited (voucher number BA013) in the Herbarium of Department of Biology, Jimma University. 100 g of powdered of root of was successively extracted using maceration technique in three solvent systems (petroleum ether, chloroform and methanol) for 72 hrs in each solvent. Each solution was filtered using cotton and Whatmann filter paper No. 3. Each of the filtrate was concentrated at reduced pressure using rotary evaporator, and was subjected to antibacterial activity test. After comparing the antibacterial activities of the crude extracts of the above mentioned solvent systems, the chloroform extract was chosen for chromatographic isolation of its constituents. Then a bulk of the powdered material (900 g) was subjected to extraction employing the same procedure, and two solvent systems (petroleum ether and chloroform).

Evaluation of antibacterial activity

Test organisms: *Staphylococcus aureus* ATCC25903, *Escherichia coli* ATCC25722, *Pseudomonas aeruginosa* DSMZ1117 and *Salmonella thyphimurium* ATCC13311 were used for antibacterial activity tests. All are standard strains obtained from the Department of Boilogy, Jimma University.

Preparation of test solutions and antimicrobial assay using disk diffusion method: Test solutions were prepared by dissolving 100 mg of each of the crude extracts in 1 ml of dimethyl sulfoxide (DMSO) to achieve final stock concentration of 100 mg/ml solution of test sample. A cell suspension of each organism was freshly prepared by tranfering isolated colonies selected from 24 hrs agar plate in to a broth and a sespention turbidity was adjusted to a 0.5 McFarland turbidity

standard (1×10^8 CFU/mL) in sterile saline solution. The solution was then diluted 1:20 to yield 5×10^5 CFU/mL [46]. The bacterial suspension (5×10^5 CFU/mL) was spread over the 90 mm Petri dishes containing Mueller Hinton agar using a sterile cotton swab. Then six mm diameter sterile discs (Whatmann No 3 paper) were placed on the surface of the inoculated Agar in Petri dishes, and 50 μ l of each test solutions were applied onto the discs. After addition of test solutions on the discs, the extract was allowed to diffuse for 5 minutes and the plates were then kept in an incubator at 37°C for 24 hrs [47]. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler. Ciprofloxacin, which is a broad spectrum antibiotic, was used for comparison. Similar procedures were used for evaluation of antibacterial activities of the pure compounds.

Isolation and characterization of compounds: The crude chloroform extract of roots of *Sida rhombifolia* was subjected to column chromatography (CC) that was packed with silica gel to isolate compounds. A glass column was packed with 100 g silica gel slurry dissolved in petroleum ether. The crude material was adsorbed onto dry of silica gel. Then the solvent was allowed to evaporate, and the dry sample adsorbed to the silica gel was applied into the column that was already packed with silica gel. TLC analyses of the crude material gave good separation of pigments on TLC plate in solvent system that was composed of petroleum ether and ethyl acetate mixture. Therefore, the mixture was used in different combinations with increasing polarity (in the ratio 100:0, 98:2, 96:4, 94:6, 92:8, 90:10%) to elute the column. A total of 123 fractions each with 40 ml were collected. Solvents were removed from the fractions under reduced pressure using rotary evaporator. The developed spots on TLC plates were visualized under UV light at 254 and 365 nm and then by exposure to iodine vapor. The fractions that showed the same TLC development profiles (color and R_f) were combined and concentrated to dryness under reduced pressure using rotary evaporator. The structures of the compounds were elucidated based on combined spectral data which include Infra Red, Nuclear Magnetic Resonance ($^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and DEPT-135) spectra data and melting point values as well as comparison of these data with data in literature. All spectroscopic analysis were carried out at Department of chemistry, Addis Ababa University.

Results and Discussion

Scientific investigations also indicated that extracts from different parts of the plant including its roots showed several types of biological activities including antibacterial activities suggesting the potential of the plant as source of new antibacterial drugs. In this work, extraction and isolation of compounds from roots of *Sida rhombifolia*, and evaluation of antibacterial activities of compounds isolated from roots of *Sida rhombifolia*.

Evaluation of antibacterial activities crude extracts and isolation compounds from roots of *Sida rhombifolia*

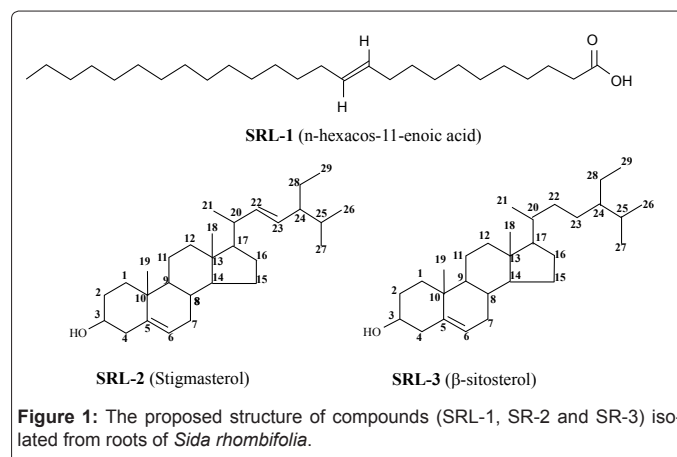
For preliminary antibacterial activity tests, 100 g of plant material (roots) was subjected to gradient extractions using maceration technique in three solvent systems; namely petroleum ether chloroform and methanol. Removal of solvents from filtrates resulted in 1.62 g, 2.04 g and 1.9 g of crude material from petroleum ether, chloroform and methanol extracts, respectively. The extracts were subjected to antibacterial activity tests using four bacterial species (*Staphylococcus aureus* ATCC25903, *Escherichia coli* ATCC25722, *Pseudomonas aeruginosa* DSMZ1117 and *Salmonella thyphimurium* ATCC13311) following the procedures described above. The antibacterial activity tests were carried out in duplicate for each crude extract against each of

the the test bacterial species, and the results are given as average mean \pm standard deviation of the duplicates observed inhibition zone (in mm) (Table 1).

Though their antibacterial activities were relatively lower than that of the reference compound (ciprofloxacin), the three crude extracts showed significant activities against all the bacterial species. The results are consistent with previous reports describing antibacterial activities of methanolic root extract *Sida rhombifolia* [24] and traditional use roots of this plant to treat bacterial infections or wounds [13]. Close observation of the data (Table 1) indicated that the activity of the methanol extract is relatively (slightly) higher than chloroform and petroleum ether extracts against *E. coli* and *S. typhimurium* (Table 1). In the case of chloroform extract, it was more active against *E. coli* (17 ± 0) and *S. aureus* (18 ± 1) but less active than against *P. aeruginosa* (13.8 ± 0) and *S. typhimurium* (14.5 ± 1.5). The activities of petroleum ether extracts were generally lower as compared to that of methanolic and chloroform extracts. Though the methanolic extract showed relatively superior antibacterial activities as compared to the chloroform and petroleum ether extracts in the preliminary antibacterial activity tests, the chloroform extract (the next more active extract) (Table 1) was selected for further analysis, i.e., for isolation of compounds using column chromatography due to the complexity of TLC profile of the methanol extract. Thus, a 900 g of the powdered plant material (root) was macerated by petroleum ether and chloroform using gradient extraction technique. 14.5 g of crude extract was obtained from chloroform extract. Then 8.0 g of the crude extract was adsorbed onto 10 g of silica gel that subsequently loaded into glass column packed with 100 g of silica gel. The column was eluted with petroleum ether and ethyl acetate mixture in different combination with increasing polarity (in the ratio 100:0, 98:2, 96:4, 94:6, 92:8, 90:10%). A total of 123 fractions each with 40 ml were collected. Some of fractions were combined based similarity of their TLC profiles. Thus, fractions 24-25 were combine to afford 0.2 g of compound (labelled as SRL-1); fractions 45-47 were also combine to give 0.26 g of pure product (labelled as SRL-2). Similarly, fractions 49-51 were also combined to afford 0.18 g of pure compound that is labelled as SRL-3.

Characterization of the isolated compounds from roots of *Sida rhombifolia*

The three pure compounds (SRL-1, SRL-2 and SRL-3) from the chloroform extract of the root of roots of *Sida rhombifolia* were characterized to be *n*-hexacos-11-enoic acid, stigmasterol and β -sitosterol, respectively (Figure 1). The compounds were characterized using spectroscopic techniques (NMR and IR spectroscopic



Extracts	Bacterial species			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>
PE extract	16 ± 1	16 ± 2	10 ± 0	16.5 ± 0.5
Chl. extract	17 ± 0	18 ± 1	13.8 ± 0	14.5 ± 1.5
Met. extract	20 ± 0	15 ± 2	16 ± 0	17.5 ± 2
Ciprofloxacin	35	32	28	30

PE: petroleum ether; Chl.: chloroform; Met.: methanol extract

Table 1: Bacterial inhibition zones (in mm) of 50 mg/ml of crude extracts of roots of *Sida rhombifolia*.

techniques). The structural elucidation was done by comparing the observed spectral and melting point data with the reported data of these compounds in the literature.

Structure elucidation of SRL-1

Compound SRL-1 was obtained colorless crystal with R_f value of 0.6 (petroleum ether and ethyl acetate, 80:20%). Its IR (KBr) spectra displayed characteristics absorption band for hydroxyl group of carboxylic acid (3407 cm^{-1}) carbonyl group of carboxylic acid (1705 cm^{-1}), and long aliphatic chain (731 cm^{-1}). The strong band at 3017 cm^{-1} represents C-H stretch of alkenes whereas the bands at 2925 and 2833 cm^{-1} indicate C-H stretching of methylene and methyl groups, respectively (Supplementary material 1). Thus, compound is probably an aliphatic acid. The $^1\text{H-NMR}$ spectrum of SRL-1 (Supplementary material 2) showed the presence of olefinic group at $\delta 5.38$ and 5.34 assigned to H-11 and H-12 respectively. Two doublets at $\delta 2.82$ and 2.79 where accounted to C-2 methylene protons adjacent to a carboxylic group. Two multiplets at $\delta 2.36$ and 2.08 both integrated for two protons can be assigned to protons attached to C-10 and C-13, respectively, which is adjacent to olefinic carbons whereas a triplet at $\delta 0.91$ was ascribed to protons at C-24. The remaining methylene protons resonated at $\delta 1.65$ (2H), 1.33 (10 H) and 1.27 (26-H). The $^{13}\text{C-NMR}$ and DEPT-135 data of SRL-1 (Supplementary material 3) presented important signals for carboxylic carbon at 179.8 (C-1), vinylic carbons at 130 (C-11) and 128.2 (C-12), for methyl carbon at 14.2 (C-26) and for methylene carbons between 34.01 and 22.71 . The DEPT-135 spectrum (Supplementary material 4) was also consistent with the $^{13}\text{C-NMR}$ data. The absence signals between $\delta 5.32$ - 2.82 (in $^1\text{H-NMR}$ spectrum) and between $\delta 128$ - 34.01 ($^{13}\text{C-NMR}$ spectrum) ruled out the existence of any carbinol carbon in the molecule. The observed IR and NMR data were found to be consistent with the reported data of *n*-hexacos-11-enoic acid. Moreover, the observed mp value (236 - 237°C) was comparable to mp value (240 - 242°C) reported for *n*-hexacos-11-enoic acid [48]. Thus, based on this observation, the chemical structure of SRL-1 was proposed to be identical with that of *n*-hexacos-11-enoic acid (Figure 1). The observed NMR data of SRL-1 and the reported data for *n*-hexacos-11-enoic acid [48], are given in (Table 2).

Structure elucidation of SRL-2

This compound was obtained as a white powder with R_f value of 0.33 (petroleum ether and ethyl acetate, 80:20%). IR (KBr) spectrum of SRL-2 has no a doublet band at/near 2850 and 2750 cm^{-1} that indicated the compound has no aldehyde functional group. The absence of strong band in the range or around 1700 - 1800 cm^{-1} also confirmed that the compound has no carbonyl group. The absence of weak bands in the range of 2000 and 1650 cm^{-1} indicated that the compound has no aromatic functional group. On the other hand, the observed stretching band at 3429 cm^{-1} indicates the presence of hydroxyl functional group. The strong band at 3007 cm^{-1} represents C-H stretch of alkenes whereas the bands at 2930 and 2858 cm^{-1} indicated C-H stretching of methylene and methyl groups. The observed IR data suggested that the compound could be an alcohol possessing a C=C double bond in its chain (Supplementary material 5). In the $^1\text{H-NMR}$ spectrum of SRL -2, the

peaks at $\delta 0.70$, 0.71 , 0.82 , 0.86 , 1.03 and 1.27 indicated the presence of protons of six methyl ($-\text{CH}_3$) groups whereas the peak at $\delta 3.55$ indicated presence of protons of a carbon attached to oxygen (hydroxyl group). The peaks at $\delta 5.06$, 5.14 and 5.38 indicated the presence of olefinic protons in SRL-2 (Supplementary material 6). The $^{13}\text{C-NMR}$ showed signals at 140.7 , 121.7 and 138.3 , 129.2 ppm which are assigned to C-5, C-6 and C-22, C-23 double bonds, respectively. The δ value at 71.8 ppm is due to C-3 β -hydroxyl group (Supplementary material 7). Totally $^{13}\text{C-NMR}$ and DEPT-135 (Supplementary material 8) spectra showed 29 and 26 signals, respectively, that can be assigned to six methyl, nine methylene, eleven methane and three quaternary carbon atoms. The observed IR and NMR data were found to be consistent with the reported data of stigmasterol [49-51]. Moreover, the observed melting point of SRL-2 (169 - 171°C) was found to be in good agreement with the reported melting point of stigmasterol (i.e. 176°C) [52]. Thus, based on these observations, the chemical structure of SRL-2 was proposed to be identical with that of the stigmasterol (Figure 1). The NMR data of SRL-2 and reported data of stigmasterol are given in (Table 3).

Structure elucidation of SRL-3

This compound was obtained as a colorless needle-like solid with R_f value of 0.30 (petroleum ether and ethyl acetate, 80:20%). Analysis of IR (KBr) spectrum of SRL-3 showed absence of a doublet band at/near 2850 and 2750 cm^{-1} indicated that the compound has no aldehyde functional group. The absence of strong band (s) around (or in the range of 1700 - 1800 cm^{-1}) confirmed that the compound has no carbonyl group. The absence of weak bands in the range of 2000 and 1650 cm^{-1} indicated that the compound has no aromatic functional group. Thus, the observed stretching band at $\delta 3432\text{ cm}^{-1}$ indicated the presence of hydroxyl functional group. The strong band at $\delta 3007\text{ cm}^{-1}$ represents C-H stretch of alkenes whereas the bands at $\delta 2930$ and $\delta 2855\text{ cm}^{-1}$ indicate C-H stretching of methylene and methyl groups. The data indicated the compound is an alcohol with C=C bond in its chain (Supplementary material 9). In the $^1\text{H-NMR}$ spectrum of SRL-3, the peaks at $\delta 0.70$, 0.71 , 0.80 , 0.82 , 0.95 and 1.03 indicated presence of protons of six methyl ($-\text{CH}_3$) groups whereas the peak at $\delta 3.55$ indicated presence of protons attached to hydroxyl group carbon. The peak at $\delta 5.37$ indicates presence of olefinic protons in the structure (Supplementary material 10). The $^{13}\text{C-NMR}$ (Supplementary material 11) has shown signals at $\delta 140.8$ and 121.7 that can be assigned to C-5, C-6 indicating C-C stretch of olefines. The value at $\delta 71.8$ is can be attributed to C-3 β -hydroxyl group. Totally, the $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data showed 29 and 26 signals, respectively, for six methyl, nine methylene, eleven methane and three quaternary carbon atoms. The observed IR and NMR data were found to be consistent with the reported data of β -sitosterol [49,51,26]. The observed melting point (136 - 138°C) was also found to be in good agreement with reported melting point of β -sitosterol (i.e., 133°C) [51-53]. Thus, based on similarities of spectral and melting point data, the chemical structure of SRL-3 was proposed to be identical with that of β -sitosterol (Figure 1) [54]. The NMR data of SRL-3 and that of β -sitosterol are given in (Table 4).

Antibacterial test of the isolated compounds

In vitro tests were carried out to evaluate antibacterial activities of the isolated compounds (SRL-1, SRL-2 and SRL-3) using Agar diffusion method and four bacterial species: *E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhimurium*. The activities of the compounds were expressed in terms of growth inhibition zones (given in mm). The growth inhibitory activities of the compounds are given in (Table 5).

Similar to that of the crude extracts, the antibacterial activities of the isolated compounds were lower than that of the reference drug

Carbon	¹³ C-NMR data of SRL-1	Reported ¹³ C-NMR data of <i>n</i> -hexacos-11-enoic acid*	¹ H-NMR data of SRL-1	Reported ¹ H-NMR data of <i>n</i> -hexacos-11-enoic acid*	DEPT-135 data of SRL-1	Nature of carbon
1	179.8	177.3	-	-	-	C
2	34.0	34.01	2.8 2, 2.79	2.77, 2.75	34.0	CH ₂
3	24.6	24.7	1.65	1.65	24.6	CH ₂
4	25.2	25.7	1.27	1.25	25.6	CH ₂
5	29.3	29.3	1.27	1.25	29.3	CH ₂
6	29.5	29.5	1.27	1.25	29.6	CH ₂
7	29.7	29.7	1.27	1.25	29.7	CH ₂
8	29.7	29.7	1.33	1.3	29.7	CH ₂
9	29.7	29.7	1.33	1.3	29.7	CH ₂
10	31.9	32.0	2.36	2.34	31.9	CH ₂
11	130	130.1	5.38	5.39	130.0	CH
12	128.2	127.9	5.34	5.32	127.9	CH
13	31.5	31.6	2.01	2.01	31.9	CH ₂
14	29.7	29.7	1.33	1.3	29.7	CH ₂
15	29.7	29.7	1.33	1.3	29.7	CH ₂
16	29.7	29.7	1.27	1.25	29.7	CH ₂
17	29.7	29.7	1.27	1.25	29.7	CH ₂
18	29.7	29.7	1.27	1.25	29.7	CH ₂
19	29.7	29.7	1.27	1.25	29.7	CH ₂
20	29.7	29.7	1.27	1.25	29.7	CH ₂
21	29.7	29.7	1.27	1.25	29.7	CH ₂
22	29.1	29.1	1.27	1.25	29.1	CH ₂
23	29.0	29.0	1.27	1.25	29.0	CH ₂
24	27.2	27.2	1.27	1.25	27.2	CH ₂
25	22.7	22.7	1.33	1.3	22.7	CH ₂
26	14.2	14.2	0.91	0.88	14.1	CH ₃

*Data from Surendra et al.

Table 2: ¹³C-NMR, DEPT-135 and ¹H-NMR data of SRL-1 along with the corresponding reported ¹³C-NMR and ¹H-NMR data of *n*-hexacos-11-enoic acid.

C. No.	¹³ C-NMR data of SRL-2	Reported ¹³ C-NMR data of stigmaterol*	DEPT-135 data of SRL-2	¹ H-NMR data of SRL-2	Reported ¹ H-NMR data of stigmaterol*	Nature of the carbon
1	37.2	37.5	37.2			CH ₂
2	31.6	31.8	31.6			CH ₂
3	71.8	71.9	71.8	3.55	3.45	CH
4	42.2	42.2	42.3			CH ₂
5	140.7	140.9	-			C
6	121.7	121.7	121.7	5.38	5.33	CH
7	31.9	31.9	31.9			CH ₂
8	31.9	32.2	31.9			CH
9	50.1	50.3	50.1			CH
10	36.5	36.6	-			C
11	21.0	21.0	21.0			CH ₂
12	39.7	39.7	39.7			CH ₂
13	42.3	42.5	-			C
14	56.7	57.0	56.7			CH
15	24.3	24.4	24.4			CH ₂
16	29.7	28.9	28.2			CH ₂
17	56.0	56.0	55.9			CH
18	12.2	12.4	12.2	0.70	0.68	CH ₃
19	19.4	19.4	19.4	1.03	0.97	CH ₃
20	40.5	40.5	40.5			CH
21	21.1	21.1	21.1	1.20	1.01	CH ³
22	138.3	138.4	138.3	5.36	5.12	CH
23	129.2	129.4	129.2	5.06	4.98	CH
24	51.2	51.3	51.2			CH
25	39.7	32.0	39.7			CH
26	19.0	19.0	19.0	0.83	0.86	CH ₃
27	21.2	21.2	21.2	0.71	0.71	CH ₃
28	25.4	25.4	25.4			CH ₂
29	12.0	12.0	11.8	0.82	0.78	CH ₃

*Data from Sammia et al.

Table 3: ¹³C-NMR, DEPT and ¹H data of SRL-2 in comparison with reported data of stigmaterol.

C. No.	¹³ C-NMR data of SRL-3	Reported ¹³ C-NMR data of β-sitosterol*	¹ H-NMR data of SRL-3	Reported ¹ H-NMR data of β-sitosterol*	Nature of carbon
1	37.2	37.3			CH ₂
2	31.6	31.6			CH ₂
3	71.8	71.8	3.5	3.45	CH
4	42.2	42.2			CH ₂
5	140.7	140.8			C
6	121.7	121.7	5.37	5.33	CH
7	31.9	31.9			CH ₂
8	31.9	31.9			CH
9	51.2	51.2			CH
10	36.5	36.5			C
11	21.1	21.1			CH ₂
12	39.7	39.8			CH ₂
13	42.3	42.3			C
14	56.7	56.8			CH
15	24.3	24.3			CH ²
16	28.2	28.2			CH ²
17	56	56.7			CH
18	12.0	12.0	0.70	0.68	CH ₃
19	19.4	19.4	0.95	0.97	CH ₃
20	36.5	36.5			CH
21	18.8	18.9	1.03	1.01	CH ₃
22	33.9	33.9			CH ₂
23	26.0	26.2			CH ₂
24	45.8	45.8			CH
25	29.2	29.1			CH
26	19.8	19.8	0.82	0.86	CH ₃
27	19.4	19.4	0.71	0.71	CH ₃
28	23.0	23.0			CH ₂
29	12.2	12.2	0.80	0.78	CH ₃

*Data from Sammia et al.

Table 4: ¹³C-NMR, DEPT-135 and ¹H-NMR data of SRL-3 in comparison with reported data of β-sitosterol.

S. No	Compounds	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>
1	SRL-1(<i>n</i> -hexacos-11-enoic acid)	13	12	8	11
2	SRL-2 (Stigmasterol)	14	12.5	9.5	13
3	SRL-3 (β-sitosterol)	13.5	11	8.5	12
	Ciprofloxacin	35	32	28	30

Table 5: Inhibition zone in mm of the isolated compounds using 50 mg/L of their solutions.

(ciprofloxacin) against all bacterial species used in the experiment, and their growth inhibition values were also comparable to each other (Table 5). Moreover, the compounds showed the least antibacterial activities against *P. aeruginosa* (Table 5 vs. Table 1). As shown above (Table 5), SRL-1 (*n*-hexacos-11-enoic acid) showed comparable antibacterial activity with that of SRL-2 and SRL-3. However, there are no reports in literature to compare and contrast our result. The antibacterial activities of SRL-2 (stigmasterol) were found to be moderate as compared to compared to the reference drug (ciprofloxacin). This observation is in good agreement with previous reports that showed low antimicrobial (or antibacterial) activities of stigmasterol against *Acetobacter* sp., *E. coli*, *S. aureus*, and *Streptococcus* sp, *P. aeruginosa* [50,54,55]. On the other hand, antibacterial activity of SRL-3 (β-sitosterol) was also found to be consistent with literature reports that discuss low/moderate antibacterial activity of β-sitosterol against several bacterial species that include *S. aureus*, *E. coli*, and *P. aeruginosa* [56-60].

Summary of Spectral Data of the Isolated Compounds

SRL-1 (colorless crystal, 0.2 g), mp 236-237°C; IR (KBr) V_{max} cm⁻¹

731, 967, 1285, 1459, 1705, 2833, 2925, 3017, 3407, ¹HNMR (400 MHz, CDCl₃): δ0.91 (3H, H-26), δ1.27 (26H, 2×CH₂), δ1.33 (10H, 5×CH₂), δ1.65 (2H, H-3), δ2.0 (2H, H-10), δ207 (2H, H-13), δ2.36 (1H, H-10) δ2.7 (2H, H-2), ¹³CNMR δ2.5.34 (1H, H-11), δ5.38 (1H, H-12), (400 Hz CDCl₃): δ14 (C-26), δ22.7 (C-25), δ24.6 (C-3), δ25.2 (C-4), δ27.2 (C-24), δ29.0 (C-23), δ29.1 (C-22), δ29.3 (C-5), δ29.5 (C-6), δ29.7 (C-11) δ31.54 (C-13), δ31.9 (C-10), δ37.2 (C-10), δ34.0 (C-26), δ128.2 (C-12), δ130.2 (C-11).

SRL-2 (White powder, 2.6 g), mp 169-171°C; IR V_{max} cm⁻¹ (KBr), 959, 1048, 1377, 1466, 1715, 2858, 2930, 3007, 3429, ¹H-NMR (400 MHz, CDCl₃): δ0.7 (3H, H-18), δ0.71 (3H, H-27), δ0.82 (3H, H-29), δ1.03 (3H, H-21), δ3.55 (1H, H-3), δ5.06 (1H, H-23), δ5.36 (1H, H-22) δ5.38 (3H, H, H-6), ¹³CNMR, (400 Hz CDCl₃): δ12 (C-29), δ12.2 (C-18), δ19.03 (C-26), δ19.4 (C-19), δ21 (C-11), δ21.1 (C-21), δ21.2 (C-27), δ24.3 (C-15), δ25.4 (C-28), δ29.7 (C-16) δ31.6 (C-2), δ31.9 (C-7, C-8), δ36.5 (C-10), δ37.2 (C-10), δ39.7 (C-12, C-25), δ40.5 (C-20), δ42.2 (C-4), δ42.3 (C-13), δ50.1 (C-9), δ51.2 (C-24), δ56.0 (C-17), δ56.7 (C-14), δ71.8 (C-3), δ127.1 (C-6), δ129.2 (C-23), δ138.3 (C-22), δ140.7 (C-5).

SRL-3 (colorless needle 0.18 g), mp 136-138°C; IR V_{max} cm⁻¹ (KBr), 943, 1055, 1388, 1633, 1710, 2855, 2930, 3007, 3432, ¹HNMR (400 MHz, CDCl₃): δ0.7 (3H, H-18), δ0.71 (3H, H-27), δ0.7 δ0.80 (3H, H-27), 7 δ0.82 (3H, H-26), δ0.95 (3H, H-29), δ1.03 (3H, H-21), δ3.5 (1H, H-3), δ5.37 (1H, H-22), ¹³CNMR, (400 Hz CDCl₃): δ12 (C-18), δ12.2 (C-29), δ18.8 (C-21), δ19.4 (C-27), δ19.8 (C-26), δ21.1 (C-11), δ23.0 (C-28), δ24.3 (C-15), δ26.0 (C-23) δ28.2 (C-16), δ31.9 (C-7, C-8), δ33.9 (C-22), δ42.2 (C-4), δ42.3 (C-13), δ45.8 (C-9), δ50.1 (C-24), δ56.0 (C-17), δ56.7 (C-14), δ71.8 (C-3), δ127.1 (C-9), δ140.7 (C-5).

Conclusions

In conclusion, three compounds (SRL-1, SRL-2 and SRL-3) were isolated from the crude acetone extract. The identities of the compounds were determined to be *n*-hexacos-11-enoic acid, stigmasterol and β-sitosterol, respectively, based on physical properties and spectroscopic (IR and NMR) data as well as literature reports. The isolation of SRL-1 (*n*-hexacos-11-enoic acid) is reported for the first time from *Sida rhombifolia*. *In vitro* test results showed that the antibacterial activities of the isolated compounds were found to be lower than the reference compound (Ciprofloxacin). When compared to each other, the antibacterial activities of the compounds were comparable to each other. But the activities of the compounds were relatively generally lowest against *P. aeruginosa*. The results were also consistent with that of the crude extracts. The observed antibacterial activities of the crude extract and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections. Thus, further test is recommended on large number of bacterial strains to decide their potential as candidates in development of antibacterial drugs.

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References

- Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. *Metabolites* 2: 303-336.
- Mishra BB, Tiwari VK (2011) Natural products in drug discovery: Clinical evaluations and investigations. *Nat Prod Med Chem* 1-62.
- Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 70: 461-477.

4. Rates SMK (2001) Plants as source of drugs. Toxic 39: 603-613.
5. Dahanukar SA, Kulkarni RA, Rege NN (2000) Pharmacology of medicinal plants and natural products. Indian J Pharmacol 32: S81-S118.
6. Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100: 72-79.
7. Dewick PM (2004) Medicinal Natural Products, a Biosynthetic Approach (2ndedn), John Wiley and Sons Ltd, Chichester, England.
8. Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 109: 69-75.
9. Choong ES (2009) Cinchona alkaloids in synthesis and catalysis, ligands, immobilization and organo-catalysis. John Wiley and Sons, Chichester, England.
10. Mabberley DJ (2000) The Plant Book (2ndedn) Cambridge University Press, Cambridge.
11. Thounaojam MC, Jadeja RN, Ramani UV, Devkar RV, Ramachandran AV (2011) *SiSida rhomboidea*. *Roxb Leaf Extract Down-Regulates Expression of PPAR γ 2 and Leptin Genes in High Fat Diet Fed C57BL/6J Mice and Retards in vitro 3T3L1 Pre-Adipocyte Differentiation*. Int J Molc Sci 12: 4661-4677.
12. Cunha AH, Merétika, Peroni N, Hanazaki N (2010) Local knowledge of medicinal plants in three artisanal fishing communities (Itapoá, Southern Brazil), according to gender, age, and urbanization. Acta Bot Bras 24: 386-394.
13. Singh N, Dubey K (2012) An ethnobotanical study of medicinal plants in Sonebhadra District of Uttar Pradesh, India with reference to their infection by foliar fungi. J Med Plant Res 6: 2727-2746.
14. Leonard DB (2008) Medicine at your feet: Healing plants of the Hawaiian kingdom Emerson.
15. Akendegue B (1992) Medicinal plants used by the Fang traditional healers in Equatorial Guinea. J Ethnopharmacol 37: 165-173.
16. Assam AJ, Dzoyem JP, Pieme CA, Penlap VB (2010) *In vitro* antibacterial activity and acute toxicity studies of aqueous-methanol extract of *Sida rhombifolia* Linn. (Malvaceae). BMC Complement Altern Med 10: 40.
17. Ragunathan M, Abay S (2009) A survey on knowledge and attitude of pharmacy, health science and medical students towards traditional medicine as well as willingness of students and doctors towards the integration of traditional and modern medicine in Gondar University. Pharmacognosy J 1: 146-153.
18. Megersa M (2011) Ethnobotanical study of medicinal plants in Wayu Tuka Wereda, East Wollega zone of Oromia region, Ethiopia. Addis Ababa Univeristy, Addis Ababa, Ethiopia.
19. Seshathri K, Thiyagarajan T (2011) Antimicrobial activity of chewing sticks of Jimma-Ethiopia against *Streptococcus pyogens*. J Phytolog 3: 34-37.
20. Rashmi RS, Uma SM, Prasanta KC (2010) Comparative *In vitro* Antimicrobial Activity Studies of *Sida rhombifolia* Linn fruit extracts. Inter J Pharm Tech Res 2: 1241-1245.
21. Thounaojam MC, Jadeja RN, Patel DK, Devkar RV, Ramachandran AV (2010) Acute and sub chronic oral toxicity of *Sida rhomboidea*. *Roxb* leaf extract. J Complementary and Integrative Medicine 7: 1.
22. Singh A, Malhotra S, Subban R (2008) Anti-inflammatory and analgesic agents from Indian medicinal plants. Intl J of Integrative Biol 3: 57-72.
23. Gupta SR, Nirmal SR, Patil RY, Asane GS (2009) Anti-arthritis activity of various extracts of *Sida rhombifolia* aerial parts. Nat Prod Res 23: 689-695.
24. Ranjan SR, Shankar MU, Kumar PS, Saiprasanna B (2011) Evaluation of antidiarrhoeal activity of *Sida rhombifolia* Linn. root. Intl Res J Pharm 2:157-160.
25. Dhalwal K, Deshpande YS, Purohit AP (2007) Evaluation of *in vitro* antioxidant activity of *Sida rhombifolia* (L.) ssp. *retusa* (L.). J Med Food 10: 683-688.
26. Bumrela SB, Naik SR (2011) Identification of β -carotene and β -sitosterol in methanolic extract of *Dipteracanthus patulus* (Jacq) nees and their role in antimicrobial and antioxidant activity. Intl J Phytomedicine 3: 204-215.
27. Narendhirakannan RT, Limmy TP (2010) *In vitro* antioxidant studies on ethanolic extracts of leaf, stem and root of *Sida rhombifolia* L. Intl J Pharma and Bio Sci 2: 1-10.
28. Mohd FF, Kadir HA, Tayyab S (2012) Bilirubin clearance in temporarily hyperbilirubinemic rats treated with aqueous extract of *Sida rhombifolia*. Life Sci J 9: 2254-2256.
29. Islam ME, Haque E, Mosaddik MA (2003) Cytotoxicity and antibacterial activity of *Sida rhombifolia* (Malvaceae) grown in Bangladesh. Phytother Res 17: 973-975.
30. Dhalwal K, Vaibhav MS, Singh B, Kakasaheb RM (2010) Hypoglycemic and hypolipidemic effect of *Sida rhombifolia* ssp. *retusa* in diabetic induced animals. Intl J Phytomed 2: 160-165.
31. Poojari R, Gupta S, Maru G, Khade B, Bhagwat S (2009) *Sida rhombifolia* ssp. *retusa* seed extract inhibits den induced murine hepatic preneoplasia and carbon tetrachloride hepatotoxicity. Asian Pac J Cancer Prev 10: 1107-1112.
32. Muanza DN, Kim bw, Euler KL, Williams L (1994) Antibacterial and antifungal activities of nine medicinal plants from Zaire. Intl J Pharmacogno 32: 337-345.
33. Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM (2002) Chemical characterization of Basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. Iran J Agri Food Chem 50: 5878-5883.
34. Rajalakshmy R, Pydi SK (2011) Cardio protective medicinal plants. Intl J Pharm Inv 1: 24-41.
35. Yan-Hong W, Bharathi A, Jadhav AN, Smillie TJ, Khan IA (2008) Structural characterization and identification of ecdysteroids from *Sida rhombifolia* L. in positive electrospray ionization by tandem mass spectrometry. Rapid Commun Mass Spectrom 22: 2413-2422.
36. Jadhav AN, Pawar RS, Avula B, Khan IA (2007) Ecdysteroid glycosides from *Sida rhombifolia* L. Chem Biodivers 4: 2225-2230.
37. <http://www.answers.com/topic/pharmacology-biological-activities-of-tagetes>
38. Jadhav AN, Swamy RC, Bharathi A, Khan IA (2007) HPTLC method for determination of 20-Hydroxyecdysone in *Sida rhombifolia* L and dietary supplements. Chromatographia 66: 797-800.
39. Islam ME, Naznin AK, Haque ME (2002) *In vitro* antibacterial activity of the extracts and a Glycoside from *Sida rhombifolia* Linn. J Med Sci 2: 134-136.
40. Chowdhury A, Ashrafal Alam M, Rahman MS, Rashid MA (2009) Phytochemical and biological investigations of *Sida Rhomboidea* Linn. Internet J Alternative Med 7
41. Prakash A, Verma RK, Ghosal S (1981) Alkaloid constituents of *Sida acuta*, *S. humilis*, *S. rhombifolia* and *S. spinosa*. Plant Med 43: 384-388.
42. Goyal MM, Rani KK (1989) Neutral constituents of the aerial parts of *Sida rhombifolia* var. *rhomboidea*. Fitoterapia 60: 163-164.
43. Goyal MM, Rani KK (1988) Effect of natural products isolated from three species of *Sida* on some gram-positive and gram-negative bacteria. J Ind Chem Soc 65: 74-76.
44. Menaka TC, Jadeja RN, Devkar RV, Ramachandran AV (2010) *Sida rhomboidea*. *Roxb* leaf extract ameliorates gentamicin induced nephrotoxicity and renal dysfunction in rats. J Ethnopharmacol 132: 365-367.
45. Muthuswamy R, Solomon MA (2009) Ethnomedical survey of folk drugs used in Bahirdar Zuria district, Northwestern Ethiopia. Ind J Trad Knowl 8: 281-284.
46. Wayne PA (2009) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 9th edition, Clinical and Laboratory Standards Institute.
47. Nascimento GF, Locatoli J, Freitas PC, Silva GL (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz J Microbiol 31: 247-256.
48. Sharma SK, Vasudev N, Ali M (2009) A new aliphatic acid from *Achyranthes aspera* Linn Roots. Ind J Chem 48: 1164-1169.
49. Pateh UU, Garba HM, Iliya I, Sule IM, Abubakar MS, et al. (2009) Isolation of stigmasterol, β -sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhizomes of *stylochiton lancifolius* pyer and kotchy (araceae). Nig J Phar Sci 7: 19-25.
50. Sahidin A, Muhammad T, Marianti M (2011) Terpenoids from the stem bark of jatropa plants and their biological activities. MAKARA SAINS 15: 106-110.
51. Kamboj A, Kumar AS (2011) Isolation of stigmasterol and β -stosterol from petroleum ether extract of aerial part of *Ageratum conyzoides* (Asteraceae). Intl J Pharm Pharmaceut Sci 3: 94-96.
52. Habib MR, Nikkon F, Rahman M, Haque ME, Karim MR (2007) Isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pak J Biol Sci 10: 4174-4176.

-
53. Sammia Y (2011) Studies on bioactive natural products of selected species of family *malvaceae*. University of Lahore, Pakistan.
54. Ahamed MK, Krishna V, Gowdr HB, Rajanaika H, Kumaraswamy HM, et al. (2007) Isolation of bacterial constituents from the Stem Bark Extract of *Grewia tiliaefolia* Vahl. Res J Med Plant 1: 72-82.
55. Tamokou JD, Kuate JR, Tene M, Julbelin T, Nwemeguela K, et al. (2011) The antimicrobial activities of extract and compounds isolated from *Brillantaisia lamium*. Iran J Med Sci 36: 24-31.
56. Beltrame FL, Ferreira AG, Cortez DA (2002) Coumarin glycoside from *Cissus sicyoides*. Nat Prod Lett 16: 213-216.
57. Mokbel MS, Hashinaga F (2005) Evaluation of the antimicrobial activity of extract from Buntan (*Citrus grandis* Osbeck). Pak J Biol Sci 8: 1090-1095.
58. Sanches NR (2005) An evaluation of antibacterial activities of *Psidium guava* (L). Braz Arch Biol Tecnol 48: 429-436.
59. Salvador MJ, Zuchi OLAD, Candido RC, Ito IY, Dias DA (2004) *In vitro* antimicrobial activities of crude extracts and isolated constituents of *A. maritime*. Pharm Biol 42: 138-148.
60. Hess SC, Brum RL, Honda NK, Cruz AB, Moretto E (1995) Antibacterial Activity and Phytochemical Analysis of *Vochysia divergens* (Vochysiaceae). J Ethnopharmacol 47: 97-100.