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Terpenoids and steroids from the stem bark of *Sesbania grandiflora* and biological studies of the plant extracts

Abstract

Phytochemical analysis of the ethyl acetate extract of the stem bark of *Sesbania grandiflora* (L.) led to the isolation of seven compounds including, a tetracyclic diterpene, kaurenoic acid (**1**). Other isolated compounds include two pentacyclic triterpenes β-amyrin (**2**) and lupeol (**3**), two steroidal ketones, stigmata-4,22-dien-3-one (**4**) and stigmast-4-en-3-one (**5**) stigmasterol (**6**) and a fatty acid, linoleic acid (**7**). Structures of these compounds were characterized by extensive NMR analysis and by comparing their spectral data with the published values. Among these compounds 1, **2**, **3**, **4** and **5** are isolated from *S. grandiflora* for the first time. Chemotaxonomic significance of the isolated compounds is also described herein. A comparative biological study for antioxidant, antimicrobial, thrombolytic and cytotoxic activities of the leaf and bark extracts of the plant was also conducted. Crude methanol extracts of both leaf and bark demonstrated strong antibacterial activity against *Bacillus megaterium* and *Aspergillus niger* as compared to the standards kanamycin and ketoconazole. The dichloromethane and ethyl acetate extract of both the bark and leaf showed very mild to moderate activities against the five microorganisms studied. The petroleum ether extract of the leaf showed no activity against most of the microorganisms studied. In the cytotoxic activity assay using brine shrimp and tamoxifen as a standard, the methanol extracts of the bark indicated the highest lethality as compared to the leaf extracts. Among all the extractives the ethyl acetate extracts of leaf showed moderate antioxidant activity using DPPH free radical scavenging method with butylated hydroxyl anisole and ascorbic acid as standards whereas different bark extracts showed very mild activities. The ethyl acetate and methanol extracts of the leaf exhibited significant thrombolytic activity as compared to the standard Streptokinase.

Keywords: *Sesbania grandiflora,* kaurenoic acid, β-amyrin, stigmata-4,22-dien-3-one, stigmast-4-en-3-one, antioxidant, cytotoxic, thrombolytic

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Introduction

Sesbania grandiflora (L.) Pers. commonly known as vegetable hummingbird, agati or hummingbird tree[1] is a small tree belonging to the family *Leguminoseae*. This plant is native to tropical Asia and is widely available in Malaysia, Indonesia, Philippines, and India.[2] It has widely been used in the herbal system of medicine to treat various diseases such as for the remedy of a large number of diseases like smallpox, inflammation, dysentery, rheumatism, leprosy, fever, gout, stomatitis and headache.[3,4] The flowers and leaf of *S. grandiflora* were described to contain anxiolytic,[5] anticancer[6] and antioxidant[7] activities. The bark extract demonstrated potent acute inflammation as well as adjuvant-induced arthritis inhibitory activity in rats.[8] Qualitative analysis of the plant is carried out using standard chemical methods. The results revealed the presence of alkaloids, carbohydrates, phenolic compounds, tannin, flavonoids and saponins in plant extracts.[9] Although *S. grandiflora* was previously studied extensively for its phytopharmacological potential, no comparative biological studies have been performed before among different extracts of the leaf and stem bark of this plant. We also report herein the isolation of seven compounds including a diterpene, two triterpenes, three steroids and, a fatty acid. The compounds are kaurenoic acid (**1**), β-amyrin (**2**) lupeol (**3**), stigmata-4,22-dien-3-one (**4**) stigmast-4-en-3-one (**5**) stigmasterol (**6**) and linoleic acid (**7**) respectively among which compounds **1**, **2**, **3**, **4** and **5** are being reported for the first time from *Sesbania grandiflora.* Previous studies claimed that kaurenoic acid (**1**) showed significant hemolytic activities,[10] whereas compounds lupeol (**3**)[11] and stigmasterol (**6**)[12] showed potential cytotoxic activities on cancer cell lines.

Material and methods

Plant materials

The plant samples were collected from Gazipur district, Dhaka, Bangladesh in May 2017. A twig containing a flower, a fruit and, a dried pod was sent to Bangladesh National Herbarium (BNH) for identification (Accession number: DACB-47383).

Chemicals and solvents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Co., USA. Silica gel and silica gel F_{254} plates were purchased from *Macherey-Nagel*, Germany. Nutrient agar media, standard disc of kanamycin and ketoconazole were purchased from Hi media, India. All the chemicals and solvents used were of analytical grades.

General experimental procedures

Buchi rotary evaporator was used to concentrate the soluble extracts. The Vacuum liquid chromatography (VLC) column was dry-packed with Kiselgel 60H under suction. Sephadex LH-20 was used to perform gel permeation chromatography. All the solvents were distilled before use. Analytical and preparative thin-layer chromatography (TLC and PTLC) were performed both on aluminum sheets and glass plates coated with silica gel (Kiselgel 60 F_{254}). The TLC spots were confirmed under UV light at 254 nm and 366 nm and also by spraying with vanillin/H2SO4 reagent followed by heating. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz Ultrashield NMR Spectrophotometer equipped with broadband and selective inverse probes. Chemical shifts are presented in δ (ppm) using Tetramethylsilane (TMS) as internal standard and coupling constants (*J*) are expressed in hertz.

Extraction for isolation

The air-dried and coarsely powdered stem barks of *S. grandiflora* (1.8 kg) were soaked in ethyl acetate (5 L) for 15 days at room temperature with occasional shaking and then double filtered using clean cotton plug. Each of the filtered extracts was concentrated with a rotary evaporator at 45°C to yield 55 g of crude extract. An aliquot of the crude extract was fractionated by vacuum liquid chromatography (VLC) using silica gel 60H. The solvent system was used in the following order: petroleum ether > petroleum ether-dichloromethane > dichloromethane-ethyl acetate > ethyl acetate > ethyl acetate-methanol, and methanol in increasing order of polarity. The VLC fractionation yielded 45 fractions.

Following TLC screening, VLC fraction No. 13 and 14 (eluted with 90% dichloromethane in petroleum ether) were subjected to gel permeation chromatography over lipophilic Sephadex (LH-20). Compound **1** (8.0 mg) and **7** (11.0 mg), were obtained from the Sephadex column fraction eluted with 70% petroleum ether in chloroform and were purified by pTLC. R_f values were found 0.33 and 0.36 respectively (in 0.5% ethyl acetate in toluene).

VLC fraction No. 16, 17 and 18 (eluted with 100% dichloromethane) were subjected to gel permeation chromatography over lipophilic Sephadex (LH-20).

Compound **2, 3**, **4** and **5** were obtained by mixing of similar Sephadex column fractions eluted with 100% chloroform and then purified by pTLC. Compound **2** and **3** (10.0 mg; Rf value: 0.51) and compound **4** and **5** were obtained as solid mixture (15.0 mg; Rf value: 0.49). The R_f values of these mixtures were 0.51 and 0.49 respectively (in 10% ethyl acetate in toluene). Compound **6** (5.5 mg) was obtained as off-white crystals, from the Sephadex column fraction eluted with 90% petroleum ether in chloroform and was purified by pTLC. The R_f value was 0.27 (in 10% ethyl acetate in toluene).

Spectroscopic data

Kaurenoic acid (**1**).[13] Off white amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (1H, m, H-1), δ 1.90 (1H, m, H-1), δ 1.47 (1H, m, H-2), δ 1.92 (1H, m, H-2), δ 1.04 (1H, m, H-3), δ 2.19 (1H, m, H-3), δ 1.09 (1H, m, H-5), δ 1.87 (2H, m, H-6), δ 1.48 (1H, m, H-7), δ 1.55 (1H, m, H-7) δ 1.09 (1H, m, H-9), δ 1.62 (1H, m, H-11), δ 1.64 (1H, m, H-11), δ 1.50 (1H, m, H-12), δ 1.63 (1H, m, H-12), δ 2.66 (1H, m, H-13), δ 1.18 (1H, m, H-14), δ 2.01 (1H, m, H-14), δ 2.08 (2H, m, H-15), δ 4.73 (1H, s, H-17), δ 4.79 (1H, s, H-17), δ 1.23 (3H, s, H-18), δ 0.94 (3H, s, H-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ 40.7 (C-1), 19.1 (C-2), 37.8 (C-3), 43.7 (C-4), 57.1 (C-5), 21.8 (C-6), 41.3 (C-7), 44.2 (C-8), 55.1 (C-9), 39.7 (C-10), 18.4 (C-11), 33.1 (C-12), 43.9 (C-13), 39.7 (C-14), 49.0 (C-15), 155.9 (C-16), 103.0 (C-17), 29.0 (C-18), 183.2 (C-19), 15.6 (C-20).

β-amyrin **(2)**.[14] Off-white crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.14 (1H, m, H-3), 5.11 (1H, t, *J* = 3.6 Hz, H-12), 0.78 (3H, s, H-23), 0.95 (3H, s, H-24), 0.82 (3H, s, H-25), 0.92 (3H, s, H-26), 1.12 (3H, s, H-27), 0.98 (3H, s, H-28), 0.86 (3H, s, H-29), 0.86 (3H, s, H-30).

Lupeol **(3)**.[15] Off white crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.14 (1H, m, H-3), 0.95 (3H, s, H-23), 0.75 (3H, s, H-24), 0.82 (3H, s, H-25), 1.01 (3H, s, H-26), 0.93 (3H, s, H-27), 0.77 (3H, s, H-28), 4.62 (br. s), 4.50 (br. s, H-29), 1.67 (3H, s, H-30).

Stigmata-4, 22-dien-3-one **(4)**.[16] White crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (1H, s, H-4), 0.71 (3H, s, H-18), 1.17 (3H, s, H-19), 1.00 (3H, m, H-21), 5.11 (1H, m, H-22), 5.03 (1H, m, H-23), 0.79 (3H, m, H-26), 0.81 (3H, m, H-27), 0.83 (3H, m, H-29).

Stigmast-4-en-3-one **(5)**.[16] White crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (1H, s, H-4), 0.71 (3H, s, H-18), 1.17 (3H, s, H-19), 0.90 (3H, d, *J*=6.6, H-21), 0.79 (3H, m, H-26), 0.81 (3H, m, H-27), 0.83 (3H, m, H-29).

Stigmasterol (**6**).[17] Off-white crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.50 (1H, m, H-3), 5.30 (1H, d, *J* = 5.4, H-6), 4.97 (1H, dd, *J* = 15.2, 8.5, H-22), 5.12 (1H, dd, *J* = 15.2, 8.5, H-23), 1.01 (3H, s, Me-18), 0.70 (3H, s, Me-19), 1.02 (3H, d , *J* = 7.4, Me-21), 0.80 (d, *J* = 6.9, Me-26), 0.84 (d, *J* = 6.3, Me-27), 0.81 (t , *J* = 7.5, Me-29).

Linoleic acid (**7**).[18] Straw colored oily liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (2H, t, J=7.5 Hz, H-2), 1.66 (2H, m, H-3), 1.27-1.33 (14H, m, H-4,5,6,7,15,16,17), 2.06 (4H, m, H-8,14), 5.35 (4H, m, H-9, 10, 12, 13), 2.79 (2H, m, H-11), 0.91 (3H, m, H-18).

Biological investigation

The leaf and bark samples of *S. grandiflora* were air-dried for several days and ground into powder separately. About 400 g of the powders from both leaf and bark parts were soaked separately in 1.2 L of methanol at room temperature for 7 days with occasional stirring and shaking. The resultant mixtures were filtered through cotton plug and concentrated with a rotary evaporator at 45°C temperature to yield the crude methanol extracts of *S. grandiflora* (MEF-L: 14.62 g and MEF-B: 13.24 g). Finally, a portion of the concentrated methanol extracts (10 g from each plant part) were fractionated by the modified Kupchan partitioning Method[19] into petroleum ether (PEF-L and PEF-B), dichloromethane (DCMF-L and DCMF-B) and ethyl acetate (EAF-L and EAF-B) fractions. Subsequent evaporation of solvents yielded 2.76 g (PEF-L), 2.85 g (DCMF-L) and 2.94 g (EAF-L) extracts from leaf part and 2.26 g (PEF-B), 2.41 g (DCMF-B) and 2.57 g (EAF-L) extracts from bark part, respectively. All the dried extracts were kept in the tight containers in the refrigerator for further studies.

Antioxidant activity

The DPPH scavenging method[20] was used to evaluate the antioxidant potential of the leaf and bark extracts of *S. grandiflora*. The calculated amount of extracts (about 1.6 mg each) were dissolved in methanol to get different concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562µg/mL) in test tubes by serial dilution technique. In each test tube, 2.0mL of the sample solution was mixed with 2.0

mL of a DPPH (1, 1-diphenyl-2-picrylhydrazyl) in methanol solution (20µg/mL) to obtain the above-mentioned concentrations. Each sample was kept in dark chamber at room temperature for about 30 minutes. The absorbance of each sample was measured against methanol as negative control by UV spectrophotometer. Butylated Hydroxy Anisole (BHA) and ascorbic acid were used ***as reference solutions as well as*** positive control. The degree of decolorization of DPPH from purple to yellow indicated the scavenging activity of the extract. The percentage of radical scavenging activity was calculated by the following equation:

Radical scavenging (%) = $[(A_0-A_1/A_0)\times100]$.

Where,

A_0 = Absorbance of the control solution (DPPH solution without sample) and

A_1 = Absorbance of the sample extracts (DPPH solution with sample).

The extract concentration that produces 50% inhibition (IC_{50}) was calculated from the plot of percentage inhibition against extract concentration. A lower IC_{50} value corresponds to a higher antioxidant activity of the extract.

Antimicrobial activity

In *vitro* antimicrobial activity of the crude methanol extracts and other fractions were assessed against two Gram-positive: *Staphylococcus aureus* (ATCC 25923) and *Bacillus megaterium* (ATCC 28318) as well as two Gram-negative bacteria; *Pseudomonus aeruginosa* (ATCC 27833) and *Escherichia coli* (ATCC 28739) and one fungal strain *Aspergillus niger*. The antimicrobial activity was tested by the standard disc diffusion method.[21] The samples were dissolved separately in a specific volume of dichloromethane or methanol depending on their solubility. Kanamycin (30µg/disc) and ketoconazole (30µg/disc) were used as standards for antibacterial and antifungal screening respectively.

Cytotoxic activity

In brine shrimp lethality bioassay,[22] adequate amount of fractions of test samples were dissolved in DMSO to obtain solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781µg/mL) by serial dilution technique in premarked glass test tubes (containing 5 mL of seawater with 10 matured shrimps in each test tube). DMSO was used as a solvent and negative control while tamoxifen served as the standard and positive control. The mortality of brine shrimp was observed after 24 hours of treatment for each of the concentrations. An approximate linear correlation was observed by plotting logarithm of concentration versus percentage of mortality in triplicate test samples.

Thrombolytic activity

For this test about 5 mL of blood was drawn from healthy human volunteers (n=5) (aged 25-40 years) who did not have a history of oral contraceptive or anticoagulant therapy. 500µL blood was transferred to each of the previously weighed and marked Eppendorf tubes (n = 8) and allowed them to form clots. 5 mL of 0.9% sodium chloride (NaCl) was added to the commercially available lyophilized S-Kinase™ (Streptokinase) vial of 15, 00,000 I.U. and mixed properly. This solution was used as a stock from which 100µL was used for in vitro thrombolytic assay.[23] Streptokinase (SK) solution was used as the standard and positive control. As a negative control, 100µL of distilled water was added to the control tube. All tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluids were discarded from each tube and the tubes were weighed again to observe the difference in weight after clot disruption.

The differences in weights taken before and after clot lysis were expressed as the percentage of clot lysis as shown below:

% of clot lysis = (weight of released clot /clot weight) × 100.

Statistical analysis

All statistical analysis was done using Microsoft Excel. Statistical values were considered significant when $P<0.05$. Antioxidant, antimicrobial, cytotoxic and thrombolytic activity was conducted in triplicate and expressed as mean ± standard deviation.

Results and discussion

Structure elucidation

Compound **1** was obtained as an off-white amorphous powder. The ^{1}H NMR spectrum (Table 1) displayed the characteristic exomethylene group at δ 4.73 s and 4.79 s and two methyl groups at δ 1.23 and 0.94. The ^{13}C NMR spectrum exhibited 20 carbons including a carbonyl carbon at δ 183.2. In the HMBC experiment (Table 1) the methyl at δ 1.23 (Me-18) and the proton δ 1.05 (H-5) showed ^3J correlation to the carbonyl carbon, thus placing the carboxyl group at C-19. The structure of the compound was established as Kaurenoic acid by analysis of NMR data including HSQC and HMBC correlations and was further confirmed by comparison of the ^{1}D NMR data published in the literature. Kaurenoic acid, a diterpene, not reported from this plant before or even from the genus *Sesbania* (Figure 1).

Biological activity

Antioxidant activity

Different organic fractions of *S. grandiflora* were subjected to evaluation of antioxidant activity. The IC_{50} value of standard BHA and ascorbic acid were 9.26±0.10µg/mL and 8.58±0.13µg/mL, respectively. Among all the extracts, ethyl acetate and methanol fractions showed significant inhibitory activity with IC_{50} values of 52.52±0.11 and 73.37±0.09µg/mL, respectively in leaf and 71.29±0.16 and 89.20±0.18µg/mL, respectively in bark part. The results are summarized in Table 2.

Antimicrobial activity

Antimicrobial activity of different fractions of *S. grandiflora* was tested against two gram-positive and two gram-negative bacterial species. During the evaluation, ethyl acetate and methanol fractions showed significant activity against *B. megaterium* with ZOI of EAF-L: 18 and MEF-L: 21 in leaf extracts and ZOI of EAF-B: 12 and MEF-B: 20 in bark extracts. The ethyl acetate and methanol fractions also showed activity against the fungal species *A. niger* with ZOI of EAF-L: 12 and MEF-L: 19 in leaf extracts and ZOI of EAF-B: 11 and MEF-B: 17 in bark extracts. The other solvent fractions showed poor to moderate inhibitory activity against all the bacterial and fungal strains. In this test, Kanamycin was used as antibacterial standard and Ketoconazole was used as antifungal standard. The results are summarized in Table 3.

Table 1 NMR spectroscopic Data of compound 1 (at 400 MHz in $CDCl_3$; in ppm, *J* in Hz)

Position	δ_C (a) (ppm)	δ_H (b) (ppm)	HSQC	HMBC
1	40.7	0.84 m, 1.90 m	40.7	--
2	19. 1	1.47m, 1.92 m	19. 1	--
3	37.8	1.04 m, 2.19m	37.8	2, 18,19
4	43.7	--	--	--
5	57.1	1.09 m	57.1	19
6	21.8	1.87 m	21.8	--
7	41.3	1.48 m, 1.55 m	41.3	--
8	44.2	---		--
9	55.1	1.09 m	55.1	--
10	39.7	---	---	--
11	18.4	1.62 m, 1.64 m	18.4	--
12	33.1	1.50 m, 1.63 m	33.1	--
13	43.9	2.66 m	43.9	--
14	39.7	1.18 m, 2.01 m	39.7	16
15	49	2.08 m	49	--
16	155.9	---	--	--
17	103	4.73 s, 4.79 s	103	--
18	29	1.23, 3H s	29	3, 4, 5,19
19	183.2	---	--	---
20	15.6	0.94, 3H s	15.6	1, 5, 9, 10

[a]Recorded at 100 MHz; [b]Proton showing long range correlation with indicated carbons

Table 2 Antioxidant activity of different fractions of *S. grandiflora*

Sample	IC_{50} value (µg/mL)*
Standard (BHA)	9.26±0.10
Standard (Ascorbic acid)	8.58±0.13
PEF-L	262.07±0.72
DCMF-L	121.63±0.09
EAF-L	52.52±0.11
MEF-L	73.37±0.09
PEF-B	253.61±0.17
DCMF-B	169.75±0.37
EAF-B	71.29±0.16
MEF-B	89.20±0.18

BHA, butylated hydroxy anisole; PEF, pet ether fraction; DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; MEF, methanol fraction; L, leaf; b, bark
*IC_{50} values represent the means±SD. of three consecutive measurements ($p< 0.05$)

Table 3 Screening of antimicrobial activity of different fractions of *S. grandiflora*

Sample Concentration		Diameter of Zone of Inhibition, ZOI (mm)				
		Gram Positive Bacteria		Gram Negative Bacteria		Fungi
		Bacillus megaterium	Staphylococcusaureus	Salmonella typhi	Escherichia coli	Aspergillus niger
Leaf extracts (400µg/disc)	PEF-L	5.67±0.58	-	-	-	6.67±0.58
	DCMF-L	10.00±1.00	9.33±0.58	-	9.67±0.58	9.33±0.58
	EAF-L	17.67±1.53	9.00±1.00	6.67±0.58	8.67±0.58	12.67±0.58
	MEF-L	22.67±0.58	11.00±1.00	11.00±1.00	11.33±1.15	19.33±0.58

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Table continued

Sample Concentration		Diameter of Zone of Inhibition, ZOI (mm)				
		Gram Positive Bacteria		Gram Negative Bacteria		Fungi
		Bacillus megaterium	Staphylococcusaureus	Salmonella typhi	Escherichia coli	Aspergillus niger
Bark extracts (400µg/disc)	PEF-B	11.67±0.58	10.00±1.00	8.33±0.58	9.00±1.00	10.33±0.58
	DCMF-B	9.67±0.58	8.00±1.00	-	6.33±0.58	8.67±0.58
	EAF-B	10.00±1.53	8.67±0.58	8.67±0.58	9.33±0.58	11.33±0.58
	MEF-B	20.67±1.15	9.33±0.58	7.33±0.58	10.67±1.15	17.00±1.00
Standards (100µg/disc)	Kanamycin	30.67±1.15	23.33±1.53	25.00±1.00	24.67±1.15	-
	Ketoconazole					28.00±1.00

PEF, pet ether fraction; DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; MEF, Methanol fraction; L, leaf; B, bark; ZOI, zone of inhibition
*ZOI values represent the means±SD. of three consecutive measurements ($p< 0.05$)

Cytotoxic activity

The median lethal concentration (LC_{50}) of the test samples after 8 hours were obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration). Ethyl acetate and methanol fractions showed very potent cytotoxic activity in both leaf (LC_{50} values were 0.6799±0.10 and 0.6564±0.04µg/mL, respectively) and bark extracts (LC_{50} values were 0.6509±0.03 and 0.6874±0.10µg/mL, respectively), in comparison to the standard (LC_{50} value 0.3019±0.05µg/mL). The results are shown in Table 4.

Table 4 Cytotoxic activity of different fractions of *S. grandiflora*

Sample	LC_{50} value (µg/mL)*
Standard (Tamoxifen)	0.3019±0.05
PEF-L	2.7865±0.46
DCMF-L	1.5941±0.26
EAF-L	0.6799±0.10
MEF-L	0.6564±0.04
PEF-B	2.5701±0.21
DCMF-B	2.2978±0.13
EAF-B	0.6509±0.03
MEF-B	0.6874±0.10

PEF, pet ether fraction; DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; MEF, methanol fraction; L, leaf; B, bark
*LC_{50} values represent the means±SD. of three consecutive measurements ($p< 0.05$)

Thrombolytic activity

The leaf and bark extracts of *S. grandiflora* showed significant thrombolytic activity. Among all the fractions, ethyl acetate and methanol soluble fraction seemed to possess the highest clot lysis activity in leaf extract (51.89±1.81% and 49.13±1.29%, respectively) and bark extract (47.23±1.16% and 45.42±1.38%, respectively) compared to the standard Streptokinase (79.68±1.43%). Distilled water showed a negligible lysis of clot (3.69±1.21%). The results are shown in Table 5.

Table 5 Thrombolytic activity of different fractions of *S. grandiflora*

Sample	% of Clot Lysis*
Negative control (Distilled Water)	3.69±1.21
Standard (Streptokinase)	79.68±1.43
PEF-L	24.75±1.16
DCMF-L	20.41±1.79
EAF-L	51.89±1.81
MEF-L	49.13±1.29
PEF-B	29.85±1.40
DCMF-B	24.47±2.92
EAF-B	47.23±1.16
MEF-B	45.42±1.38

PEF, pet ether fraction; DCMF, dichloromethane fraction; EAF, ethyl acetate fraction; MEF, methanol fraction; L, leaf; B, bark
*% of Clot lysis values represent the means±SD. of three consecutive measurements ($p< 0.05$)

Chemotaxonomic Significance

Compounds **1** and **2** are the first isolation from the *Sesbania* genus, and the first report of compound **3** in this species. Compounds **1** and **2** were previously isolated from the genus *Copaifera langsdorffii*[24] and

Caesalpinia bonduc[25] within the same family. Compound **3** was isolated from another *Sesbania* species *Sesbania aegyptiaca*.[26] This might be considered as further chemical markers to distinguish *S. grandiflora* from other *Sesbania* species. Compounds **4** and **5** are reported for the first time in Leguminoseae family, previously isolated from several plant families i.e., Asteraceae,[27] Compositae,[28] Simarubaceae[29,30] and Scrophulariaceae.[31] Therefore, steroids **4** and **5** may be an important addition of the Leguminoseae family as chemotaxonomic markers. Compounds **6** and **7** were isolated from the same species earlier.[32,33]

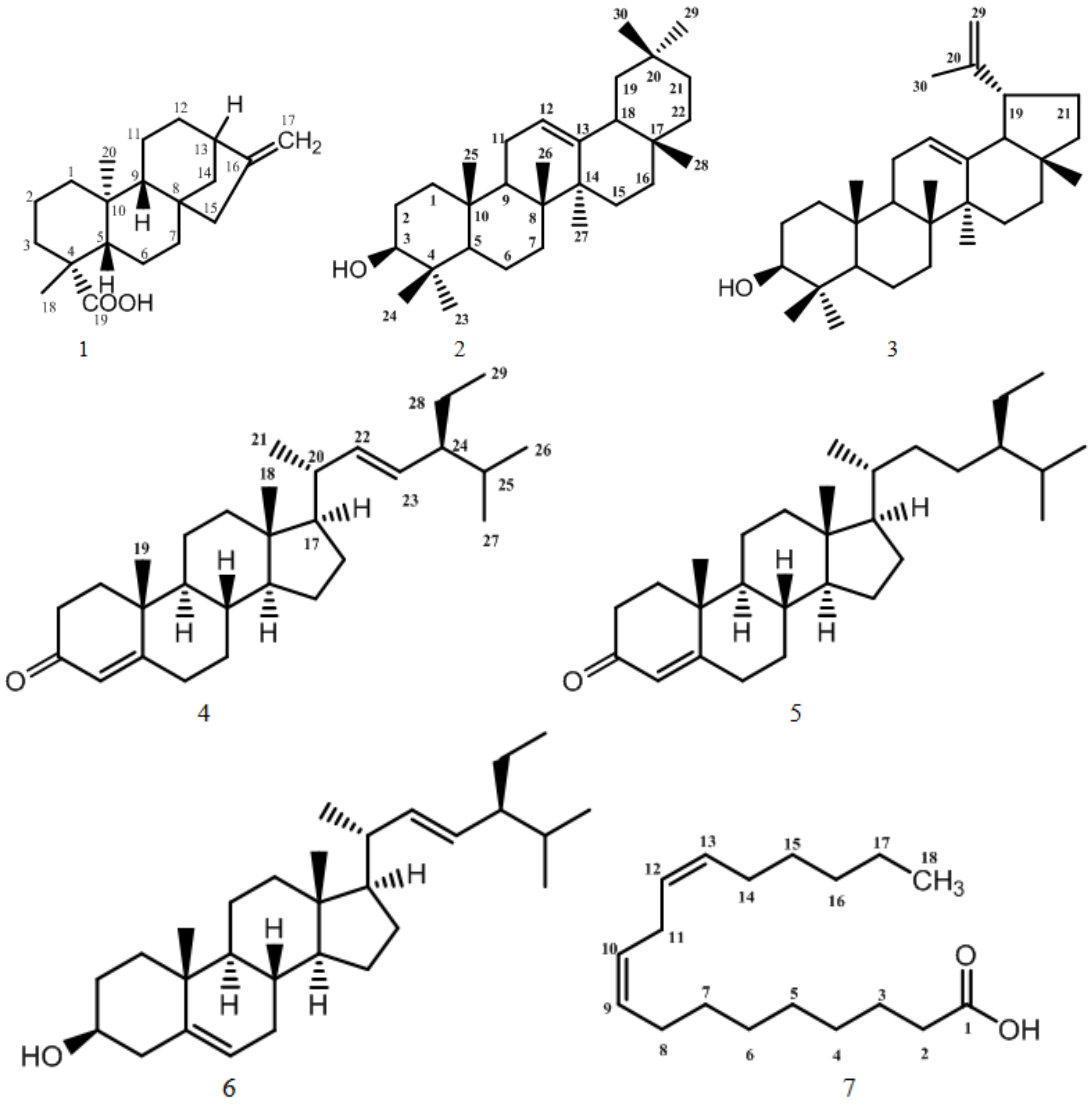

Figure 1 Compounds (1-7) isolated from *S. grandiflora*.

Conclusion

Among the isolated compounds, kaurenoic acid (**1**), β-amyrin (**2**) lupeol (**3**), stigmata-4,22-dien-3-one (**4**) stigmast-4-en-3-one (**5**) and stigmasterol (**6**) are being reported for the first time from *Sesbania grandiflora*. From the biological investigation among various fractions of leaf and bark of *S. grandiflora* (L.), it is evident that ethyl acetate and methanol extracts have significant antioxidant, antibacterial, cytotoxic and thrombolytic activities. It is strongly believed that the detailed information as reported in this study might be used as additional data for future extensive research, development, and utilization of *S. grandiflora* in various diseases. Further work is to be carried out to explore the other chemical constituents and biological activity of pure compounds.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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