

Chemical and Biological Investigations of *Albizia chinensis* (Osbeck.) Merr.

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ABSTRACT: A total of five compounds, namely lupeol (1), α -amyrin (2), stigmasterol (3), β -sitosterol (4) and 7,3'-dimethoxyluteolin (5) were isolated from a methanol extract of *Albizia chinensis* stem bark. The structures of the isolated compounds were established using nuclear magnetic resonance (NMR) spectroscopic studies, as well as using comparative thin layer chromatography (co-TLC) with standards. The methanol extract of the *A. chinensis* stem bark and its *n*-hexane-, carbon tetrachloride-, chloroform- and aqueous-soluble partitionates were evaluated for antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and phosphomolybdenum total antioxidant assays, where butylated hydroxytoluene (BHT) and ascorbic acid were used as standards. The carbon tetrachloride-soluble fraction contained a moderate amount of phenolic compounds (60.76 ± 0.45 mg of GAE/g of extract) and it exhibited significant free radical-scavenging activity ($IC_{50} 10.21 \pm 0.84 \mu\text{g ml}^{-1}$). A positive correlation was observed between the total phenolic content and the total antioxidant capacity of *A. chinensis*. The general toxicity was determined using a brine shrimp lethality bioassay, where the carbon tetrachloride ($LC_{50} 0.608 \pm 0.19 \mu\text{g ml}^{-1}$) and hexane ($LC_{50} 0.785 \pm 0.26 \mu\text{g ml}^{-1}$) soluble partitionates had considerable bioactive properties. The crude extract and its chloroform- and aqueous-soluble fractions displayed significant antimicrobial activity against eighteen microorganisms with inhibition zones ranging from 7.0 mm to 26.0 mm.

Keywords: *Albizia chinensis*, lupeol, α -amyrin, stigmasterol, β -sitosterol, 7,3'-dimethoxyluteolin

1. INTRODUCTION

Albizia chinensis (Osbeck.) Merr. (Synonyms: *Acacia stipulata* DC., *Albizia marginata* Lam. Merr.; Bengali: Chakua koroi), which belongs to the Fabaceae family, is a flowering tree that is native to south-eastern Asia but is invasive in Hawaii and Samoa. In Bangladesh, the plant is distributed throughout most districts. Traditionally, an infusion of the bark is used as a lotion for cuts,

scabies and other skin diseases. The aqueous extract of the bark causes uterine contraction. The extract of the aerial parts has spasmogenic and diuretic activities in accordance to Medicinal Plants Database of Bangladesh. The aqueous extract of the *A. chinensis* plant demonstrates higher anti-proliferative activity than the standard anti-cancer drug, cisplatin.¹ Previous phytochemical studies with *A. chinensis* revealed the presence of a number of cytotoxic triterpenes and flavonoids.^{2,3}

As a part of our continuing investigation into the medicinal plants from Bangladesh,^{4,5} we studied the methanol extract of the *A. chinensis* stem bark, as well as its organic- and aqueous-soluble fractions, for the total phenolic content, total antioxidant capacity using the phosphomolybdenum assay, free radical-scavenging activity, antimicrobial screening and brine shrimp lethality for the first time. Furthermore, we also reported the isolation of lupeol (**1**), α -amyrin (**2**), stigmasterol (**3**), β -sitosterol (**4**) and 7,3'-dimethoxyluteolin (**5**) from the *A. chinensis* methanol extract.

2. EXPERIMENTAL

2.1 General Experimental Procedures

Structural elucidation of the isolated compounds was carried out using nuclear magnetic resonance (NMR) spectroscopy. The ¹H NMR spectra were recorded using a Bruker AMX-500 (500 MHz) instrument in CDCl₃ and the δ values for the ¹H data were referenced to the residual non-deuterated solvent signals. All solvents were of analytical grade. Preparative thin layer chromatography (PTLC)⁶ and thin layer chromatography (TLC) were carried out using Merck Si gel F₂₅₄ pre-coated plates. Spots were visualised by spraying the plates with vanillin sulphuric acid followed by heating for 5 min at 110°C. All solvents used in this study were reagent grade.

2.2 Plant Materials

A. chinensis stem bark was collected in mid-2011 from the Dhaka University campus. A voucher specimen (DUSH-5386) has been deposited into the Dhaka University Salar Khan Herbarium.

2.3 Extraction, Fractionation and Purification

Collected plant materials were cleaned, chopped into small pieces, sun-dried and ground into a coarse powder. Approximately 600 g of the powdered material was soaked in 2.5 l of methanol at room temperature for several days.

The extract was first filtered through a fresh cotton bed and then filtered through Whatman (#1) filter paper. The filtrate was then concentrated with a rotary evaporator at 40°C–50°C under reduced pressure to provide a crude methanol extract (20 g).

An aliquot (3 g) of the concentrated methanol extract was then subjected to vacuum liquid chromatography.⁷ The column was packed with silica gel (Kiesel gel 60H) under vacuum. After applying the sample to the column, compounds were eluted with n-hexane, followed by mixtures of n-hexane and ethyl acetate, and methanol mixtures in order of increasing polarity to provide 22–100 ml fractions. Compound **1** (6.0 mg) was isolated as a colourless crystalline mass from the vacuum liquid chromatography (VLC) fraction of the methanol extract eluted with 15% ethyl acetate in n-hexane. The fraction eluted with 30% ethyl acetate in n-hexane provided compound **2** (3.0 mg). Similar VLC fractions eluted with 20% ethyl acetate and 15% ethyl acetate in n-hexane, yielding compounds **3** (8.0 mg) and **4** (10.0 mg), respectively.

The VLC fraction eluted with 35% ethyl acetate in n-hexane was further subjected to Gel Permeation Chromatography (GPC)⁸ using lipophilic Sephadex (LH-20), which afforded compound **5** (3.0 mg) as a yellow amorphous powder.

Another aliquot (5.0 g) of the concentrated methanol extract was partitioned using the modified Kupchan method.⁹ The resultant partitionates were evaporated to dryness with a rotary evaporator to yield n-hexane- (HXSf, 1500 mg), carbon tetrachloride- (CTCSf, 1500 mg), chloroform- (CSf, 250 mg) and aqueous- (AQSF, 750 mg) soluble fractions. The residues were stored in a refrigerator until further use.

2.4 Properties of the Isolated Compounds

Lupeol (**1**): colourless crystals; ¹H NMR (500 MHz, CDCl₃): δ 4.68 (1H, br. s, H_a-29), 4.56 (1H, br. s, H_b-29), 3.17 (1H, dd, J = 11.2, 4.8 Hz, H-3), 1.65 (3H, s, H₃-30), 1.03 (3H, s, H₃-27), 0.96 (3H, s, H₃-26), 0.95 (3H, s, H₃-25), 0.83 (3H, s, H₃-24), 0.79 (3H, s, H₃-23) and 0.76 (3H, s, H₃-28).

α-Amyrin (**2**): white amorphous powder; ¹H NMR (CDCl₃, 500 MHz): δ 5.34 (1H, t, J = 3.5 Hz, H-12), 3.19 (1H, dd, J = 11.0, 5.6 Hz, H-3), 1.03 (3H, s, H₃-27), 1.02 (3H, s, H₃-26), 0.96 (3H, s, H₃-28), 0.93 (3H, s, H₃-25), 0.90 (3H, bs, H₃-30), 0.82 (3H, s, H₃-23), 0.79 (3H, d, H₃-29) and 0.78 (3H, s, H₃-24).

Stigmasterol (**3**): white, needle-shaped crystals; ¹H NMR (CDCl₃, 500 MHz): δ 3.52 (1H, m, H-3α), 5.35 (1H, d, J = 8.2 Hz, H-6), 0.67 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.91 (3H, d, J = 6.8 Hz, Me-21), 5.27 (1H, dd, J = 8, 15.2

Hz, H-22), 5.18 (1H, dd, J = 7.2, 15.2 Hz, H-23), 0.85 (3H, d, J = 6 Hz, Me-26), 0.83 (3H, d, J = 6 Hz, Me-27) and 0.82 (3H, t, J = 6.5 Hz, Me-29).

β -sitosterol (**4**): colourless crystals; ^1H NMR (CDCl_3 , 500 MHz): δ 3.52 (1H, m, H-3), 5.35 (1H, m, J = 6.0 Hz, H-6), 0.68 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.93 (3H, d, J = 6.4 Hz, Me-21), 0.83 (3H, d, J = 7.2 Hz, Me-26), 0.82 (3H, d, J = 7.2 Hz, Me-27) and 0.85 (3H, t, J = 8.0 Hz, Me-29).

7,3'-dimethoxyluteolin (**5**): yellow amorphous powder; ^1H NMR (500 MHz, CDCl_3): δ 12.96 (1H, s, OH), 7.47 (1H, d, J = 2.3, H-2'), 7.46 (1H, dd, J = 8.4, 2.3 Hz, H-6'), 7.03 (1H, d, J = 8.4, H-5'), 6.56 (1H, s, H-3), 6.48 (1H, d, J = 2.3, H-8) and 6.37 (1H, d, J = 2.3, H-6).

2.5 Biological Assays

2.5.1 Total phenolic content

The total phenolic content of the extracts was determined with the Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd.¹⁰

2.5.2 DPPH free radical scavenging assay

Following the method developed by Brand-Williams et al.,¹¹ the antioxidant activity of the methanol extract and its sub-fractions was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

2.5.3 Phosphomolybdenum antioxidant assay

The total antioxidant activity of the extract was evaluated with the phosphomolybdenum assay method.¹²

2.5.4 Brine shrimp lethality bioassay

This technique was applied to determine the general toxic properties of the plant extract using the method of Meyer et al.¹³ and *Artemia salina* in a one-day *in vivo* assay. Vincristine sulphate was used as a positive control.

2.5.5 Antimicrobial screening

Anti-microbial screening was carried out following the method developed by Bauer et al.¹⁴

2.5.6 Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as the mean \pm standard deviation.

3. RESULTS AND DISCUSSION

3.1 Chromatographic Analysis

Repeated chromatographic separations and purification of the VLC fractions of a crude methanolic extract from *A. chinensis* stem bark provided a total of five compounds (1–5). The structures of the isolated compounds were determined to be lupeol (1),¹⁵ α -amyrin (2),¹⁶ stigmasterol (3),¹⁷ β -sitosterol (4)¹⁸ and 7,3'-dimethoxyluteolin (5)³ using extensive NMR spectral analysis, in comparison with published values and co-TLC with standards.

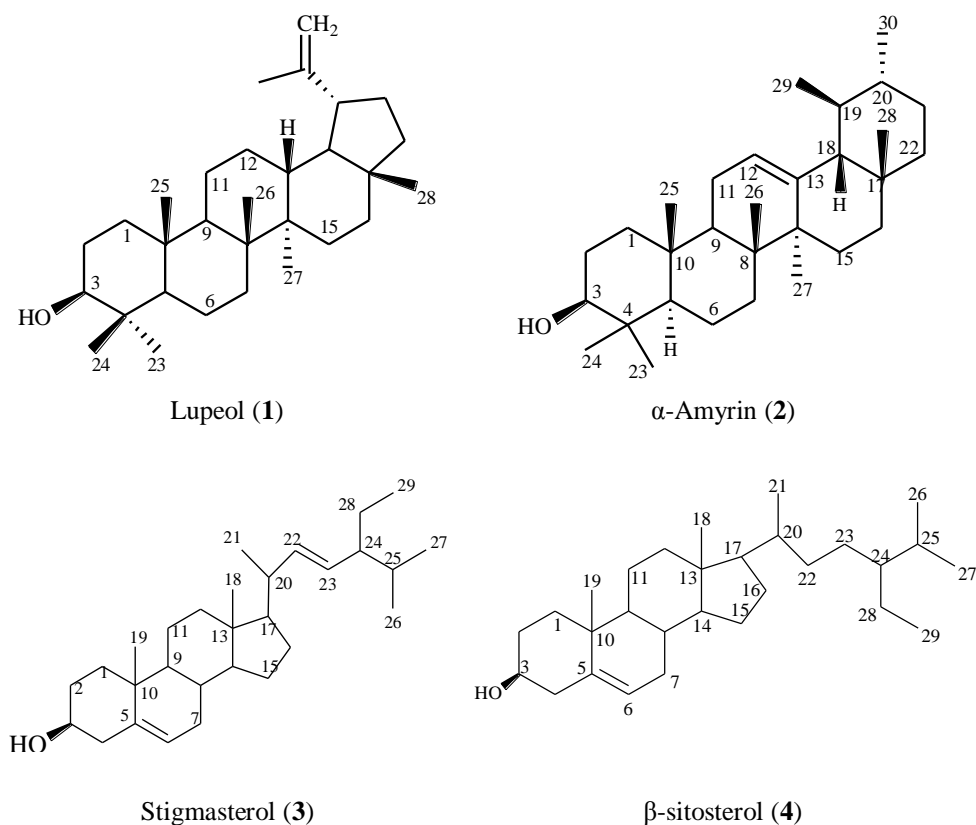


Figure 1: Structure of the compounds (continued on next page).

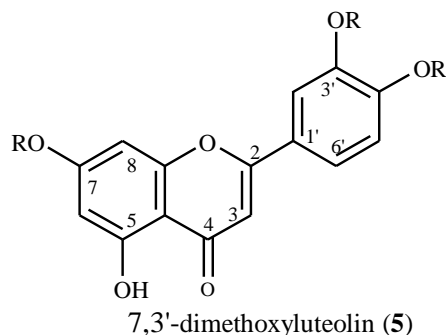


Figure 1: (continued).

3.2 Total Phenolic Content, Free Radical-scavenging Activity and Total Antioxidant Capacity of *A. chinensis*

In the DPPH free radical-scavenging assay, the carbon tetrachloride-soluble fraction had maximum free radical-scavenging activity ($IC_{50} = 10.21 \pm 0.84 \mu\text{g ml}^{-1}$) when compared to ascorbic acid ($IC_{50} = 5.8 \pm 0.21 \mu\text{g ml}^{-1}$). This prominent free radical-scavenging property may be correlated to its high phenolic content ($60.76 \pm 0.45 \text{ mg of GAE/g of sample}$) or due to synergistic activity from various chemical entities present in the extract. A positive correlation was observed between total phenolic content and total antioxidant activity of *A. chinensis* (Table 1).

3.3 Brine Shrimp Lethality Bioassay

In the brine shrimp lethality bioassay, the lowest LC_{50} ($0.608 \pm 0.19 \mu\text{g ml}^{-1}$) value was obtained with the carbon tetrachloride-soluble fraction, whereas vincristine sulphate exhibited an LC_{50} value of $0.451 \mu\text{g ml}^{-1}$. The n-hexane-soluble fraction also revealed significant cytotoxic potential ($LC_{50} = 0.785 \pm 0.26 \mu\text{g ml}^{-1}$). This suggested the presence of potent bioactive components in the crude extract (Table 1). Previous investigations on the total phenolic content, free radical-scavenging and cytotoxic activities on other species of the same genus (*Albizia*) support the above findings.^{19, 20, 21}

Table 1: Total antioxidant capacity, total phenolic content, free radical-scavenging activity and cytotoxicity of *A. chinensis*.

Sample	Total phenolic content (mg of GAE/g of dried extract)	Free radical-scavenging activity (IC ₅₀ µg ml ⁻¹)	Total antioxidant capacity (mg of ascorbic acid/100 g of plant extract)	Brine shrimp lethality bioassay LC ₅₀ (µg ml ⁻¹)
Vincristine sulphate	–	–	–	0.451 ± 0.004
BHT	–	27.5 ± 0.54	–	–
Ascorbic acid	–	5.8 ± 0.21	–	–
ME	3.98 ± 0.22	21.68 ± 0.21	0.64 ± 0.61	2.51 ± 0.41
HXSf	2.38 ± 0.56	150.33 ± 0.45	0.364 ± 0.52	0.785 ± 0.26
CTCSF	60.76 ± 0.45	10.21 ± 0.84	0.79 ± 0.25	0.608 ± 0.19
CSF	52.34 ± 0.64	18.8 ± 0.73	0.741 ± 0.45	0.98 ± 0.39
AQSF	5.03 ± 0.22	37.64 ± 0.15	0.481 ± 0.15	2.17 ± 0.22

ME = Methanol crude extract; HXSf = Hexane-soluble fraction; CTCSF = Carbon tetrachloride-soluble fraction; CSF = Chloroform-soluble fraction; AQSF = Aqueous-soluble fraction; BHT = Butylated hydroxytoluene.

3.4 Antimicrobial Activity of *A. chinensis*

Various *A. chinensis* extracts were evaluated for antimicrobial activity against five gram positive bacteria, eight gram negative bacteria and five fungi at 400 µg disc⁻¹. Different test samples of *A. chinensis* demonstrated inhibition zones ranging from 7.0 mm to 26.0 mm (Table 2). The maximum inhibition zone at 26.0 mm was demonstrated against *Sarcina lutea* by chloroform- and aqueous-soluble fractions and the methanolic crude extract. The aqueous-soluble fraction also revealed a 25.0 mm inhibition zone against *Salmonella typhi*. In addition, the crude extract inhibited the growth of *Aspergillus fumigatus* by 18.0 mm (Table 2). Similar antimicrobial activity was demonstrated against *Albizia adianthifolia* and *Albizia julibrissin*, two species of the Fabaceae family.^{19,22}

Table 2: Antimicrobial activity of *A. chinensis* test samples (400 µg disc⁻¹).

Test microorganisms	Diameter of inhibition zone (mm)					
	ME	HXSf	CTCSF	CSF	AQSF	Ciprofloxacin (30 µg disc ⁻¹)
<i>Bacillus cereus</i>	11.0 ± 0.12	–	7.5 ± 0.23	13.0 ± 0.92	12.0 ± 0.32	45.0 ± 2.01
<i>B. megaterium</i>	10.0 ± 0.43	–	8.0 ± 1.56	11.0 ± 0.23	10.0 ± 0.43	42.0 ± 1.17
<i>B. subtilis</i>	10.0 ± 0.56	–	9.0 ± 1.34	10.0 ± 0.61	10.0 ± 1.13	42.0 ± 0.73
<i>Staphylococcus aureus</i>	15.0 ± 0.57	–	–	20.0 ± 1.45	17.0 ± 1.3	42.0 ± 0.23
<i>Sarcina lutea</i>	26.0 ± 0.16	–	17.0 ± 0.45	26.0 ± 1.11	26.0 ± 0.92	42.0 ± 0.56
<i>Escherichia coli</i>	13.0 ± 0.54	–	8.0 ± 0.81	17.0 ± 0.45	21.0 ± 0.33	42.0 ± 0.43
<i>Pseudomonas aeruginosa</i>	17.0 ± 0.36	–	–	20.0 ± 0.31	18.0 ± 0.45	42.0 ± 1.11
<i>Salmonella Typhi</i>	17.0 ± 1.21	–	11.0 ± 0.17	20.0 ± 1.15	25.0 ± 0.41	45.0 ± 0.73
<i>S. Paratyphi</i>	15.0 ± 0.68	–	6.0 ± 0.32	20.0 ± 0.55	18.0 ± 1.56	47.0 ± 2.33
<i>Shigella boydii</i>	12.0 ± 0.87	–	8.0 ± 0.66	9.0 ± 1.9	9.0 ± 0.11	34.0 ± 0.58
<i>Sh. dysenteriae</i>	9.0 ± 0.73	–	–	–	6.0 ± 0.83	42.0 ± 0.22
<i>Vibrio mimicus</i>	–	–	–	–	–	40.0 ± 0.45
<i>V. parahaemolyticus</i>	18.0 ± 1.56	–	14.0 ± 1.1	19.0 ± 2.12	20.0 ± 0.21	35.0 ± 0.44
<i>Candida albicans</i>	7.0 ± 0.67	–	10.0 ± 0.21	12.0 ± 0.67	13.0 ± 0.32	38.0 ± 0.49
<i>Saccharomyces cerevisiae</i>	10.0 ± 1.12	–	9.0 ± 0.53	11.0 ± 1.43	14.0 ± 0.21	38.0 ± 0.11
<i>Aspergillus niger</i>	7.0 ± 0.43	–	10.0 ± 0.48	14.0 ± 0.33	11.0 ± 0.54	37.0 ± 0.33
<i>A. fumigatus</i>	18.0 ± 1.37	–	11.0 ± 0.22	–	8.0 ± 0.48	39.0 ± 0.26
<i>A. ustus</i>	14.0 ± 0.43	–	9.0 ± 0.13	12.0 ± 0.16	9.0 ± 0.65	38.0 ± 0.34

ME = Methanol crude extract; HXSf = Hexane-soluble fraction; CTCSF = Carbon tetrachloride-soluble fraction; CSF = Chloroform-soluble fraction; AQSF = Aqueous-soluble fraction.

4. CONCLUSION

In this study, the isolation and characterisation of lupeol (**1**), α -amyrin (**2**), stigmasterol (**3**), β -sitosterol (**4**) and 7,3'-dimethoxyluteolin (**5**) from the methanolic crude extract of *A. chinensis* have been reported. The crude extract and its partially purified fractions demonstrated strong DPPH free radical-scavenging activity, cytotoxicity in a brine shrimp lethality bioassay and antimicrobial activity. However, none of the isolated compounds could be tested for bioactivity due to sample scarcity. This plant may be used for further systematic chemical and biological studies to isolate the active components responsible for the potential bioactivity.

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