Phytochemical and Biological Investigation of *Bridelia tomentosa* Blume Growing in Bangladesh

Adeeba Anjum[1,2], Md. Ruhul Kuddus[1], Md. Zakir Sultan[3], Abdullah Al Mansur[4], Choudhury M. Hasan[1] and Mohammad A. Rashid[1]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh
[3]Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh
[4]Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Kudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

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ABSTRACT: *Bridelia tomentosa* Blume is a small evergreen tree which has enormous folklore applications in the treatment of colic, traumatic injury, epidemic influenza and neurasthenia. The main purpose of this study was to isolate bioactive compounds from the stem bark of *B. tomentosa* growing in Bangladesh. Extensive chromatographic separation and purification of the methanolic extract of stem bark of *B. tomentosa* led to the isolation of four compounds. The purified constituents were identified as friedoolean-5(6),14(15)-dien-3-one (**1**), *β*-taraxerol (**2**), D_4-stigmasterone (**3**) and lupeol (**4**) by extensive analysis of NMR spectroscopic data. While compound **1** is appeared to be new, the other compounds (**2**-**4**) have been isolated for the first time from this plant. *β*-taraxerol (**2**) demonstrated significant cytotoxic activity against the brine shrimp *Artemia salina* and moderate to strong antimicrobial efficacy with the highest inhibitory potential against *Salmonella* Typhi (zone of inhibition = 21.3 mm) and *Sarcina lutea* (zone of inhibition =20.8 mm). In conclusion, *B. tomentosa* has been found to be a rich source of secondary metabolites and thus it may be studied further in order to isolate of more bioactive constituents.

Key words: *Bridelia tomentosa*, Triterpenes, Steroids, Cytotoxicity, Antimicrobial

INTRODUCTION

Bridelia tomentosa Blume (Bengali name: Khy, Serai, Family: Phyllanthaceae) is a large tree found in the woodland areas of Sylhet, Srimangal and Chattogram as well as in Dinajpur in Bangladesh. The plant also grows in India, China, Philippines and Northern Australia.[1] Leaves of this plant have beneficial effect for traumatic injury. Bark has astringent property and roots are used in influenza and neurasthenia.[2] Previous phytochemical investigation of the fruits of *B. tomentosa* led to isolation of quercetin, tamarixetin, *β*-amyrin, ethylgallate, gallic acid, friedelin etc. Two flavonoid glycosides have been isolated from the leaves of *B. tomentosa*.[3,4]

Although there are several reports on the ethnopharmacological studies of *Bridelia* species, a few articles have been published regarding the phytochemical and biological investigations of *B. tomentosa*. Therefore, in continuation of our previous works on medicinal plants of Bangladesh[5-7], including on *Bridelia*[8,9] species, we have examined the methanol extract of stem bark of *B. tomentosa* for isolation and characterization of the secondary metabolites and subsequent evaluation of cytotoxic and antimicrobial properties of these pure compounds and we, here in, report the results of our investigation.

Correspondence to: Mohammad A. Rashid
E-mail: r.pchem@yahoo.com

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MATERIAL AND METHODS

Plant materials. Stem bark of *B. tomentosa* was harvested from Khagrachari district of Bangladesh in February, 2007. The identity of the plant was confirmed in Bangladesh National Herbarium, Mirpur, Dhaka-1216, where a voucher specimen has been deposited with the accession number of DACB-31377.

Extraction and preparation of plant extract. The powdered stem bark of *B. tomentosa* (575 g) was soaked in 1.5 L methanol for one week. Filtrations of the plant extract were done by using fresh cotton bed, and finally by Whatman No. 1 filter paper. The filtrate was concentrated with the help of a rotary evaporator at 40ºC and at reduced pressure to yield a gummy mass.

Isolation of chemical constituents. For purification of chemical compounds, an aliquot (20 g) of the crude extract of *B. tomentosa* was subjected to VLC column (14 cm length and 10 cm in diameter) over silica gel 60H. The sample from the VLC column was eluted with petroleum-ether, followed by mixtures of petroleum-ether and ethyl acetate in order of increasing polarities and lastly with methanol. After TLC screenings, the VLC fractions with identical TLC features were mixed together. VLC fractions labeled as 4A and 4B; 5B and 6A; 7B and 8A; and 12A, 12B and 13A being similar were combined separately. Preparative TLC of these mixtures using toluene and ethyl acetate provided compound **1** (8.04 mg) from fractions 4A+4B, compound **2** (5.11 mg) from fractions 5B+6A, compound **3** (6.29 mg) from fractions 7B+8A and compound **4** (6.31 mg) from fractions 12A+12B+13A.

Properties of isolated compounds (1-4)

Friedoolean-5(6),14(15)-dien-3-one(**1**): Colorless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (1H, m, H-6), 5.56 (1H, dd, *J* = 8.2, 3.6 Hz, H-15), 1.23 (3H, s, Me-23), 1.22 (3H, s, Me-28), 1.16 (3H, s, Me-29), 1.08 (3H, s, Me-27), 1.02 (3H, s, Me-24), 0.98 (3H, s, Me-26), 0.95 (3H, s, Me-30), 0.81 (3H, s, Me-25).

β-taraxerol (**2**): Colorless gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.52 (1H, dd, *J* = 8.0 and 3.5 Hz, H-15), 3.17 (1H, dd, *J* = 10.05, 3.5 Hz, H-3), 1.07 (3H, s, Me-26), 0.96 (3H, s, Me-25), 0.93 (3H, s, Me-30), 0.91 (3H, s, Me-29), 0.89 (3H, s, Me-24), 0.89 (3H, s, Me-23), 0.80 (3H, s, Me-27), 0.78 (3H, s, Me-28).

D_4-stigmasterone (**3**): White amorphous; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.76 (1H, s, H-4), 5.13 (1H, dd, *J* = 15.1, 8.3 Hz, H-22), 5.01 (1H, dd, *J* = 15.0, 8.3 Hz, H-23), 1.01 (3H, s, Me-21), 0.89 (3H, d, *J* = 4.6 Hz, Me-27), 0.85 (3H, s, Me-19), 0.81 (3H, t, *J* = 6.6 Hz), 0.80 (3H, d, *J* = 4.6 Hz, Me-26), 0.71 (3H, s, Me-18).

Lupeol (**4**): Crystalline powder; mp 215-216 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.68 (1H, s, H-29a), 4.56 (1H, s, H-29b), 3.22 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 2.30 (1H, m, H-19), 1.67 (3H, s, Me-30), 1.02 (3H, s, Me-26), 0.96 (3H, s, Me-23), 0.94 (3H, s, Me-27), 0.83 (3H, s, Me-25), 0.78 (3H, s, Me-24), 0.75 (3H, s, Me-28).

Cytotoxic activity. Brine shrimp lethality bioassay[10,11] was employed to evaluate the cytotoxic potential of the test samples using vincristine sulfate (VS) and dimethyl sulfoxide (DMSO) as positive and negative control, respectively. The LC_{50} and LC_{90} of the test compounds were obtained by plotting the percentage of shrimp killed against the logarithm of the test sample concentration.

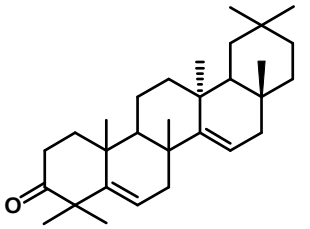
Antimicrobial activity. Antimicrobial activity of the test samples was determined by the disc diffusion method.[12]

RESULTS AND DISCUSSION

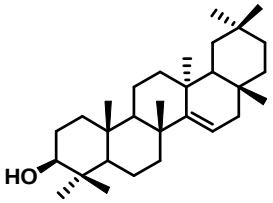
A total of four compounds, friedoolean-5(6),14(15)-dien-3-one (**1**), *β*-taraxerol (**2**), D_4-stigmasterone (**3**) and lupeol (**4**) (Figure 1) were isolated from the methanolic extract of the stem bark of *B. tomentosa* by repeated chromatographic separation and purification over silica gel. The chemical structures of these compounds were elucidated by analysis of their NMR spectral data and comparison with published values.

Compound **1** was characterized as friedoolean-5(6),14(15)-dien-3-one by studying of its ^{1}H NMR

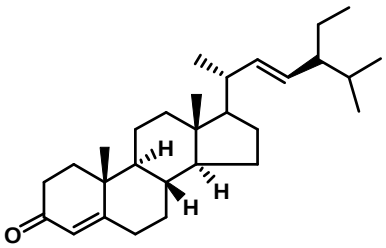
data and comparison with those of related compounds.[13-16] The ^{1}H NMR spectrum of compound **1** showed eight singlets each of three proton intensity at δ 0.81, 0.95, 0.98, 1.02, 1.08, 1.16, 1.22 and 1.23 attributable to eight methyl groups present in the molecule.[10] The spectrum further displayed two olefinic proton signals at δ 5.68 (1H, m) and 5.56 (1H, dd, *J*= 8.2 and 3.6 Hz) which could be assigned to H-6 and H-15, respectively of a pentacyclic triterpenoid skeleton.[13-15] Comparison of the ^{1}H NMR spectral data with reported values[13-16] of related triterpenoids suggested the occurrence of a pentacyclic triterpenoid skeleton. Based on the above NMR data as well as by comparison with the chemical shifts of protons of structurally related triterpenes in the literatures[13-16], compound **1** was tentatively characterized as friedoolean-5(6),14(15)-dien-3-one. To the best of our knowledge, this study represents the first report of friedoolean-5(6),14(15)-dien-3-one (**1**) from any nature and synthetic source.



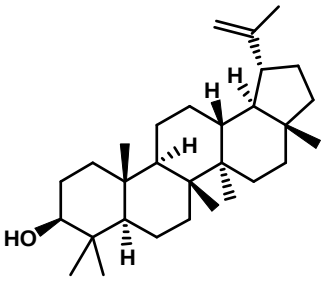
Friedoolean-5(6),14(15)-dien-3-one (**1**)



β-taraxerol (**2**)



D_4-stigmasterone (**3**)



Lupeol (**4**)

Figure 1. Friedoolean-5(6),14(15)-dien-3-one (**1**), *β*-taraxerol (**2**), D_4-stigmasterone (**3**) and lupeol (**4**) isolated from *B. tomentosa*.

The ^{1}H NMR spectrum of compound **2** showed eight three-proton singlets at δ 0.78, 0.80, 0.89, 0.89, 0.91, 0.93, 0.96 and 1.07, which were assigned to the methyl group protons at Me-28, Me-27, Me-23, Me-24, Me-29, Me-30, Me-25 and Me-27, respectively. The double doublet (*J* = 8.0, 3.5 Hz) of one proton intensity centered at δ 5.52 was assigned to the olefinic proton at C-15. Another double doublet (*J* = 10.5, 3.5 Hz) centered at δ 3.17 could be assigned to the oxymethine proton at C-3. The large couplings (*J* = 10.5, 3.5 Hz) of H-3 with the vicinal methylene protons at C-2 demonstrated a *beta* orientation of the hydroxyl group at C-3. The above information suggested the presence of a typical taraxaren-type pentacyclic triterpene skeleton and they were found to be in close agreement to those published for *β*-taraxerol.[15] Therefore, the identity of compound **2** was confirmed as *β*-taraxerol.

The ^{1}H NMR spectrum of compound **3** displayed resonances for six methyl groups, two of which were doublets at δ 0.80 (d, *J* = 4.6 Hz) and 0.89 (d, *J* = 4.6 Hz), associated to an isopropyl group. Three resonances for methine protons were observed at δ 5.76 as a singlet, 5.13 as a double doublet (dd, *J* =

15.1, 8.3 Hz) and 5.01 also as a double doublet (dd, *J* =15.0, 8.3 Hz). These were characteristics signals of H-4, H-22 and H-23 in a D_4-stigmasterone type carbon skeleton. Other spectral features were in close agreement to those of D_4-stigmasterone.[17] So, compound **3** was identified as D_4-stigmasterone.

Compound **4** showed a ^{1}H NMR spectrum with a double doublet (*J* = 11.2, 4.8 Hz) at δ 3.22, which is distinctive for an oxymethine proton at C-3 in a triterpene nucleus. The ^{1}H NMR spectral data of compound **4** were in close agreement with the published values for lupeol.[18,19] Therefore, compound **4** was characterized as lupeol (Figure 1). The identity of compound **4** as lupeol was further confirmed by co-TLC with an authentic lupeol previously isolated in our laboratory.

However, this is the first report of isolation of *β*-taraxerol, D_4-stigmasterone and lupeol from *B. tomentosa*.

Cytotoxicity of the test samples was performed by brine shrimp lethality bioassay.[10,11] In this bioassay, a marine micro crustacean specimen, *Artemia salina* Leach (brine shrimp), was used as the target organism to detect cytotoxicity of plant extracts. As shown in Table 1, the LC_{50} and LC_{90} values obtained from the best-fit line slope were found to be as 7.71 µg/ml and 99.31 µg/ml, respectively for compound **2** isolated from *B. tomentosa* as compared to the LC_{50} for positive control (vincristine sulfate, 0.45 µg/ml). Here, the percent mortality was found to increase gradually with the increase in concentration of the test sample. Brine shrimp lethality test is a very simple screening tool, which has a good correlation with the antitumor activity of test samples.[10] The US National Cancer Institute (NCI, USA) established that, there is a significant correlation between the brine shrimp lethality and *in vitro* growth inhibition of human solid tumor cell lines. Thus, this bioassay is a very important pre-screening tool for antitumor drug research.[20,21]

The natural products from plant origin have more chemical scaffolds than that of the synthetic ones, which means they have appreciable benefits in supplying new leads in commercial drug discovery program.[22] Hence, the main target or aim of our studies was to use the plant extract efficiently and effectively of this diversity. In recent times, the researchers have switched to plants for the search of prospective antimicrobial compounds. The multi-drug resistant strains of the pathogens to the presently used antibiotics is another reason for the search of new antimicrobial agents. So, many medicinal plants have been extensively explored globally for their

Table 1. Effect of pure compound 2 isolated from the stem bark of *B. tomentosa* on brine shrimp nauplii.

Conc. (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
400	2.602	100	7.71	99.31
200	2.301	100		
100	2.000	90		
50	1.699	90		
25	1.398	80		
12.5	1.097	60		
6.25	0.797	50		
3.125	0.495	20		
1.563	0.194	30		
0.781	-0.107	10		

Table 2. Results of the antimicrobial screening of the compound 2.

Test organisms	Diameter of zone of inhibition (mm)	
Gram positive bacteria	Compound **2** (50 µg/disc)	Kanamycin (30 µg/disc)
Bacillus cereus	19.1	35
B. megaterium	16.7	35
B. subtilis	14.9	36
Staphylococcus aureus	16.4	32
Sarcina lutea	20.8	27
Gram negative bacteria		
Escherichia coli	19.1	25
Pseudomonas aeruginosa	16.9	20
Salmonella Typhi	21.3	22
S. Paratyphi	19.7	27
Shigella boydii	19.8	27
S. dysenteriae	19.6	25
Vibrio mimicus	17.2	25
V. parahemolyticus	18.4	20
Fungi		Griseofulvin (20 µg/disc)
Aspergillus niger	15.8	20
Candida albicans	14.2	18
Saccharomyces cerevisiae	12.7	19

antimicrobial potential.[23-26] In the present study, compound **2** showed significnt antibacterial effect with zone of inhibition ranging from 14.9-21.3 mm (Table 2) at a concentration of 50 µg/disc. The maximum zone of inhibition (21.3 mm) was displayed by compound **2** against *S.* Typhi.

During antifungal screening, the zone of inhibition produced by the test sample, compound **2** was between 12.7-15.8 mm signifying moderate to strong activity compared to standard griseofulvin with the zone of inhibition 18-20 mm. Compound **2** showed the uppermost antifungal property against *A. niger* (zone of inhibition = 15.8 mm).

CONCLUSION

Chromatographic separation and purification of the stem bark extract of *B. tomentosa* afforded four compounds identified as friedoolean-5(6),14(15)-dien-3-one (**1**), *β*-taraxerol (**2**), D_4-stigmasterone (**3**) and lupeol (**4**), while compound **1** has been proposed as a new compound and compounds **2-4** are the first report of their isolation from *B. tomentosa.* Purified compound **2** showed moderate cytotoxicity against brine shrimp nauplii and strong antibacterial activity against a series of test microorganisms. Since, *B. tomentosa* is rich source of secondary metabolites, this can be considered as a potential source of antimicrobial and cytotoxic agent in the development of new therapeutic molecules.

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