Terpenoids and Coumarin from *Bursera serrata* Wall.

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ABSTRACT: Two terpenoids, β-amyrin (**1**) and β-sitostenone (**2**) and a coumarin, scopoletin (**3**) were isolated from petroleum ether and dichloromethane soluble extracts of the stem bark of *Bursera serrata* Wall. The structures of the isolated compounds **1-3** were established by extensive spectroscopic studies as well as by comparison with published data. Although compounds **1-3** have been isolated from various plant species previously, this is the first report of the occurrence of these compounds from *B. serrata*. The crude extractives exhibited weak antimicrobial activity and cytotoxicity.

Key words: *Bursera serrata*, Burseraceae, β-amyrin, β-sitostenone, scopoletin, antimicrobial, cytotoxicity

INTRODUCTION

Bursera serrata Wall. ex Colebr. belonging to the family Burseraceae (Syn. *Protium serratum*; Bengali name- Niyar, Hajna, Neul, Laljoyna, Chitrica, Hiliabhadi, Gutgutia) is an evergreen resinous small sized tree, distributed, mostly in hilly areas, deciduous and semievergreen forests in Bangladesh, Native India and the Philippines. It produces aromatic oil and edible fruits[1]. *Bursera* species showed anti-inflammatory,[2] anti-tumor[3] and agglutinating and immobilizing activities.[4] To the best of our knowledge, no phytochemical study has been carried out on *B. serrata*. As a part of our continuing studies with medicinal plants of Bangladesh, we investigated *B. serrata* and we, herein, report the isolation and identification of β-amyrin (**1**), β-sitostenone (**2**) and scopoletin (**3**) from *B. serrata*.

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MATERIALS AND METHODS

General. ^{1}H NMR spectra were acquired using Ultra Shield Bruker DPX 300 or 400 NMR instrument using $CDCl_3$ as solvent. The chemical shifts are reported in ppm with respect to TMS or residual non deuterated solvent signals.

Plant Material. Stem bark of *B. serrata* was collected from National Botanical Garden at Dhaka, Bangladesh in April 2004. The plant was taxonomically identified by Dr. Mahbuba Khanam, Principal Scientific Officer, at Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (accession number-DACB No. 30,733). The bark was first sun dried and then ground into a coarse powder using a grinding machine.

Extraction and isolation. The air dried and powdered plant material (0.5 kg) was successively extracted with a Soxhlet apparatus using petroleum ether (60-80^{o}C) which was followed by dichloromethane and methanol. All three extracts were filtered off and then evaporated by using Buchi rotavopour individually to get gummy concentrates of the crude extracts.

The petroleum ether soluble extract (2.0 g) was chromatographed over silica gel (Kieselgel 60, mesh 70-230) and the column was eluted with petroleum ether-ethyl acetate and ethyl acetate-methanol mixtures of increasing polarities to give a total of 50 fractions, each 25 ml. Compound **1** (10 mg) was isolated as colorless mass from fraction eluted with 7% ethyl acetate in petroleum ether and by preparative thin layer chromatography using 5% ethyl acetate in toluene, with few drops of acetic acid.

The dichloromethane extract (2.58 g) was fractionated by column chromatography in the similar fashion to get 40 fractions, each 25 ml. Compound **2** (8 mg) was isolated from fraction eluted with 10 % ethyl acetate in petroleum ether by PTLC over silica gel using 10% ethyl acetate in toluene. Similarly, compound **3** (8 mg) was obtained from column fraction eluted with 40 % ethyl acetate in petroleum ether by PTLC over F_{254} silica gel using 3% methanol in chloroform as the developing solvent.

β-amyrin (**1**): colorless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 (1H, t, *J* = 3.7, H-12), 3.23 (1H, dd, *J* = 1.0, 5.0, H-3), 1.07, 1.00, 0.99, 0.95 (each 3H), 0.80 (3H × 2) and 0.79 (3H × 2).

β-sitostenone (**2**): yellowish mass; ^{1}H NMR (400 MH_Z, $CDCl_3$): δ 5.74 (1H, s, H-4), 1.19 (3H, s, H_3-19), 0.93 (3H, d, *J* = 6.6 Hz, H_3-21), 0.85 (3H, t, *J* = 7.2 Hz, H_3-29), 0.84 (3H, d, *J* = 6.8 Hz, H_3-26), 0.82 (3H, d, *J* = 6.8 Hz, H_3-27), 0.72 (3H, s, H_3-18).

Scopoletin (**3**): white gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, *J* = 9.4 Hz, H-4), 6.92 (1H, br.s, H-5), 6.85 (3H, s, H-8), 6.25 (1H, d, *J* = 9.4 Hz, H-3), 6.10 (1H, br. s, OH-7), 3.25 (3H, br.s, OMe-6)

Bioassays. The antimicrobial activity of the extracts was determined by the disc diffusion method[5,6] against a number of gram positive and gram negative bacteria at a concentration of 500 µg/disc (Table 1). The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. In this investigation, Kanamycin (30 µg/disc) was used as the reference standard.

Table 1. Antimirobial activity of the extracts of *B. serrata* (500 µg/disc) and Kanamycin (30 µg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)		
	PE	DCM	KAN
Gram positive bacteria			
Bacillus cereus	--	9.22 ± 1.26	21.22 ± 0.33
B. megaterium	--	8.33 ± 1.67	22.34 ± 1.25
B. subtilis	8.23 ± 1.12	9.56 ± 0.87	23.34 ± 0.54
Staphylococcus aureus	8.29 ± 0.98	8.48 ± 1.25	23.37 ± 1.67
Sarcina lutea	8.46 ± 1.28	10.01 ± 1.11	20.37 ± 0.29
Gram negative bacteria			
Escherichia coli	--	8.24 ± 0.36	23.33 ± 1.20
Pseudomonas aeruginosa	8.19 ± 0.87	9.22 ± 1.26	22.37 ± 1.05
Salmonella paratyphi	8.36 ± 1.12	8.33 ± 1.34	20.31 ± 1.29
S. typhi	9.34 ± 1.29	11.26 ± 1.25	20.34 ± 1.87
Shigella boydii	--	8.47 ± 1.24	20.37 ± 0.67
Sh. dysenteriae	8.37 ± 0.67	--	22.39 ± 1.12
Vibrio mimicus	8.45 ± 0.33	--	21.37 ± 0.99
V. parahemolyticus	8.12 ± 1.54	8.37 ± 1.26	22.33 ± 1.29
Fungi			
Candida albicans	8.35 ± 1.65	--	20.37 ± 1.37
Aspergillus niger	8.46 ± 1.26	--	20.64 ± 0.91
Sacharomyces cerevisiae	8.33 ± 1.36	13.33 ± 1.31	20.22 ± 1.57

The diameters of zone of inhibition are expressed as mean ± SD (n=3); PE - petroleum ether extract; DCM - dichloromethane extract; KAN – Kanamycin; "--" Indicates no activity; a diameter of zone of inhibition less than 6 mm was considered as inactive

For cytotoxicity screening, DMSO solution of the plant extract was applied against *Artemia salina* for 24 hours *in vivo* assay.[7,8] For this experiment 4 mg of the plant extract was dissolved in DMSO and by serial dilution technique, solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/ml were prepared. After 24 hrs, the median lethal concentration LC_{50} of the test sample was obtained by ploting percentage the shrimps killed against the logarithm of the sample concentrations.

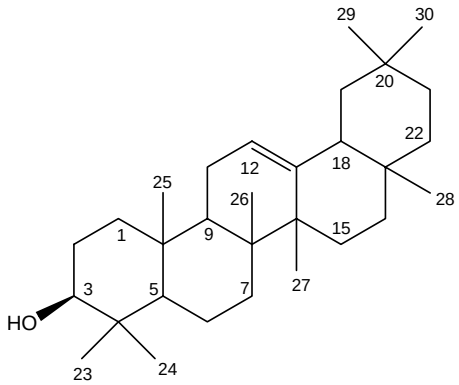
Statistical analysis. For each of the extracts, three samples were prepared for each of the bioassays. The zone of inhibition and LC_{50} were calculated as mean ± SD (n=3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS AND DISCUSSION

Extensive chromatographic separation and purification of the petroleum ether and dichloromethane soluble extracts of the stem bark of *B. serrata* provided a total of three compounds (**1**-**3**), the structures of which were elucidated by ^{1}H NMR analysis.

The ^{1}H NMR spectrum of compound **1** displayed the characteristic olefinic proton resonance as a triplet (J = 3.7 Hz) at δ 5.12 and the oxymethine proton signal as a double doublet (J = 11.0, 5.0) at δ 3.23. In addition, the ^{1}H NMR spectrum showed signals for eight methyl groups at δ 1.07, 1.00, 0.99, 0.95, 0.80 (3H × 2) and 0.79 (3H × 2). These ^{1}H NMR data was consistent with a β-amyrin[9] type carbon skeleton for **1**. Comparison of these data with the published values[9] as well as Co-TLC with authentic sample established the identity of **1** as β-amyrin. β-amyrin has previously been reported from many plants.[10] However, this is the first report of its isolation from *B. serrata.*

The ^{1}H NMR spectrum of compound **2** displayed an olefinic proton singlet at δ 5.74 (H-4). The spectrum also revealed singlets at δ 1.19 and 0.72 (each 3H, s) assignable to two tertiary methyl groups at C-10 (H_3-19) and C-13 (H_3-18), respectively. The three proton doublets at δ 0.93 (J = 6.6 Hz), 0.84 (J = 6.8 Hz) and 0.82 (J = 6.0 Hz) were demonstrative of methyl groups at C-20 (H_3-21) and C-25 (H_3-26, H_3-27), respectively. A three proton triplet (J = 7.2 Hz) at δ 0.85 was observed for another methyl group at C-28 (H_3-29). On this basis, the structure of the compound **2** was determined as β-sitostenone, an isomer of β-sitosterone (**4**). The identity of compound **2,** was confirmed by comparison of its ^{1}H NMR spectral data with that published values for β-sitostenone.[11]



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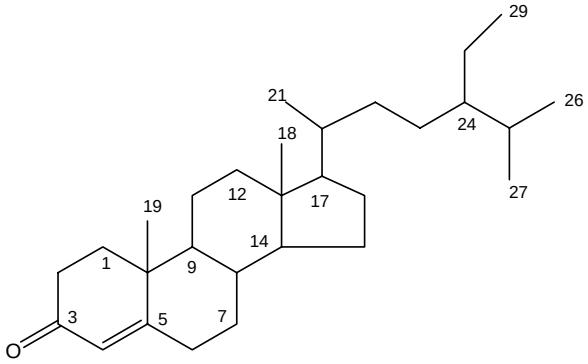

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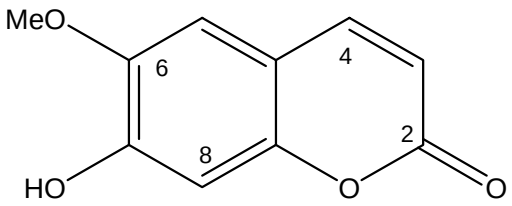

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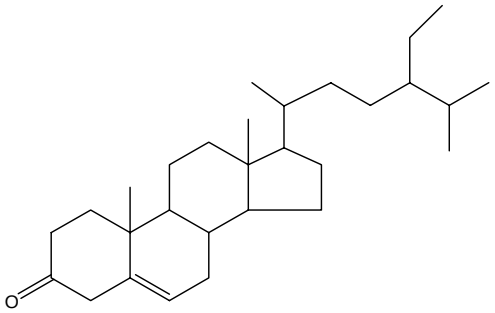

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The ^{1}H NMR spectrum of **3** displayed signals characteristics of a 6,7-dioxygenated coumarin. Thus, it showed two doublets at δ 6.27 (1H, d, *J* = 9.5 Hz) and 7.60 (1H, d, *J* = 9.5 Hz) typical of H-3 and H-4, respectively of the pyrone ring in a coumarin nucleus. The presence of two aromatic proton singlets at δ 6.92 and 6.85 were attributable to H-5 and H-8, respectively. The signal for H-8 was broadened due to long range coupling with H-4 and a three-proton singlet appeared at δ 3.96 was assigned to a methoxyl group at C-6. On this basis, compound **3** was identified as scopoletin. The identity of **3** as scopoletin was confirmed by comparing its ^{1}H NMR spectral data with literature values[12] and by Co-TLC with authentic sample. This is the first report of occurrence of these compounds from *B. serrata*.

In case of antimicrobial screening, the petroleum ether and dichloromethane soluble extracts showed the average zone of inhibition 8-9 mm and 8-13 mm (Table 1), respectively. The dichloromethane soluble extract exhibited mild inhibitory activity against *S. typhi* and *S. lutea* having the zone of inhibition 11 and 10 mm, respectively. At the same time, the petroleum ether soluble extract showed very weak inhibitory activity against the bacterial growth. In case of fungal strains, only the growth of *S. cerevisiae* was moderately inhibited by the dichloromethane soluble extract (13 mm) of *B. serrata*.

The dichloromethane extract was subjected to brine shrimp lethality bioassay.[7,8] The LC_{50} value of the extract was found to be 9.64 µg/ml, while the reference standard vincristine sulphate showed the LC_{50} value 0.44 µg/ml.

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REFERENCES

1. Huq, A.M. and Hasan, H. 1987. *Flora of Bangladesh* (Burseraceae).
2. Carretero, M.E., López-Pérez, J.L., Abad, M.J., Bermejo, P., Tillet, S., Israel, A. and Noguera, P.B. 2008. Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. *J. Ethnopharmacol.* **116**, 11-15.
3. McDoniel, P.B., and Cole, J.R. 1972. Antitumor activity of *Bursera schlechtendalii* (burseraceae): isolation and structure determination of two new lignans. *J. Pharma. Sci.* **61**, 1992-1994.
4. Huacuja, R.L., Delgado, N.M., Carranco, L.A., Reyes, L.R. and Rosado, G.A. 1990. Agglutinating and immobilizing activity of an ethanol extract of *Bursera fagaroides* on human and other mammalian spermatozoa. *Arch. Invest. Med.* **21**, 393-398.
5. Barry, A. L. 1980. *Procedures for testing antimicrobial agents in agar media,* In*: Antibiotics in Laboratory medicines* (V. Lorian. Ed), Williams and Wilkins Co., Baltimore, USA, pp. 1-123.
6. Bauer, A.W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standard single disc method. *Am. J. Clin. Pathol*. **45,** 493-496.
7. McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active constituents. *Planta Med.* **45**, 31-32.
8. Meyer, B. N., Ferringni, N.R., Puam, J. E., Lacobsen, L. B., Nicols, D. E. and McLaughilin, J.L. 1982. Brine Shrimp: A convenient general bioassay for active constituents *Planta Med.* **45**, 31-32.
9. Ercil, D., Sakar, M.K., Olmo, E.D. and Feliciano, A.S. 2004. Chemical constituents of *Linaria aucheri*. *Turk. J. Chem.* **28**, 133-139.
10. *Dictionary of Natural Products*, CD-ROM, version 9.2. Chapman & Hall, 2001.
11. Gaspar, E.M.M and Neves, H.J.C.D. 1993. Steroidal constituents from mature wheat straw. *Phytochemistry* **34**, 523-527.
12. Begum, T., Rahman, M.S. and Rashid M.A. 2007. Phytochemical and Biological investigations of *Phyllanthus reticulatus*. *The Dhaka University J. Pharma. Sci.* **5**, 5-7.