Antibacterial and Cytotoxic Constituents from *Bridelia verrucosa* Haines Growing in Bangladesh

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ABSTRACT: Fractionation and purification of methanolic extract of stem bark of the *Bridelia verrucosa* growing in Bangladesh afforded glochidonol (**1**), brassicasterol (**2**), friedelin (**3**), 5*β*-24S-ethylchoestane-3*β*-ol (**4**), stigmasterol (**5**), ketooleanane (**6**), 5-5'-dihydroxy sesamine (**7**) and pinoresinol (**8**). Compounds **1**, **2**, **3**, **4**, **6** and **8** are the first report of their isolation from the plant while compound **7** appear to be a new isolate from natural source. The isolated compounds exhibited cytotoxic activity against brine shrimp nauplii having significant LC_{50} and LC_{90} values and moderate to strong antimicrobial activity against 13 Gram positive and Gram negative bacterial strains and 3 fungi. Compound **8** showed highest inhibition of growth of microorganisms with zone of inhibition of 16.7 mm against *Shigella dysenteriae*. Compounds **1-4** and **6-8** also revealed moderate free radical scavenging activity, in DPPH assay.

Key words: *Bridelia verrucosa*, triterpenes, lignans, steroids, antimicrobial, cytotoxicity, free radical scavenging

INTRODUCTION

Bridelia verrucosa Haines (Synonym: *B. montana* Willd., *B. sikkimensis* Gehrmann, Local name: Ghiyai; Family: Phyllanthaceae) is a large shrub or straggling tree without thorns. The plant is widely distributed in Chittagong Hill Tracts, Bangladesh.[1,2] The root and bark are frequently used as astringent in Bombay and Goa in India. The plant has been widely known for its anthelmintic property.[1,3,4] Previous phytochemical studies with leaves of the plant showed the presence of sitosterol, its glucoside and hexacosanol.[4]

As part of our continuing studies on *Bridelia* species growing in Bangladesh[5,6] we isolated and identified 8 compounds from the methanolic extract of stem bark of *B. verrucosa*, and studied the biological activities of the isolated compounds.

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MATERIALS AND METHODS

Plant material. The stem bark of *B. verrucosa* was collected from the village of Panchouri, Khagrachhori District in February, 2007 and identified in Bangladesh National Herbarium, where voucher specimen has been deposited (Accession No. DACB-31376).

Materials and equipment. Column chromatography was carried out over silica gel (70-230 mesh, E-Merck) and Sephadex LH-20 (20-100 µm, Sigma-Aldrich Chemicals). Vacuum liquid chromatography (VLC) was conducted on silica gel (Kieselgel 60H). TLC and preparative TLC were run on precoated silica gel plates 60 F_{254} (Aluminium sheets, E-Merck, Germany). Melting points were measured on a hot stage melting point apparatus (PIC, India) and are uncorrected. 1D NMR spectra were recorded on Brucker 400 and 500 MHz spectrometers in solvents and chemical shifts are reported with respect to residual non-deuterated solvent signals.

Extraction and isolation. The air dried and powdered stem bark of *B. verrucosa* (550 g) was soaked in 1.5 liter methanol for 7 days and filtered through fresh cotton bed followed by Whatman filter paper number 1. The filtrate was concentrated using rotary evaporator at 40°C under reduced pressure. A portion of the concentrated methanolic crude extract (20 g) was subjected to solvent-solvent partitioning following the modified Kupchan method,[7] to yield, *n*-hexane (3.5 g), carbon tetrachloride (1.24 g), chloroform (1.6 g) and aqueous (11.4 g) soluble fractions. An aliqus portion (320 mg) of the *n*-hexane soluble fraction was subjected to gel permeation chromatography over Sephadex (LH-20) eluted with *n*-hexane-dichloromethane-methanol (2:5:1). Thirty sub-fractions were collected and those with similar TLC features were combined. After evaporation of solvents at room temperature, sub-fractions 11-18, gave white crystals, which were washed with *n*-hexane to remove the adhering impurities and recrystallized from *n*-hexane and ethyl acetate to afford compound **1** (7.23 mg). Repeated preparative TLC of sub-fractions 19-23 over silica gel using toluene-ethyl acetate (97:3) provided compound **2** (6.78 mg). The residual coloring material left after obtaining compound **1** was subjected to re-chromatography over silica gel [1.1 cm (ID) × 55 cm (L)] using *n*-hexane-ethyl acetate gradient system to obtain 50 sub-fractions and sub-fractions with identical spots on TLC were combined and kept for further investigation. At room temperature the solvents of sub-fractions 1-4 evaporated off to afford transparent crystals, which were washed with *n*-hexane to remove the impurities and recrystallized from *n*-hexane and ethyl acetate to give compound **3** (5.3 mg). Sub-fractions 5-9 and 34-43 were subjected to repeated preparative TLC over silica gel using toluene-ethyl acetate (95:5) to provide compound **4** (7.14 mg) and **5** (6.5 mg), respectively.

A portion of the carbon tetrachloride soluble fraction (270 mg) was subjected to column chromatography packed with silica gel [2 cm (ID) × 84 cm (L)]. The column was eluted initially with petroleum ether followed by petroleum ether-ethyl acetate and ethyl acetate-methanol gradients to obtain 150 sub-fractions 20 ml each. The sub-fractions giving identical TLC features were bulked together. Evaporation of solvents from sub-fractions 8-12 at room temperature afforded transparent crystals. The crystals were washed with *n*-hexane to remove the colored impurities and recrystallized from *n*-hexane and ethyl acetate to yield compound **6** (7.53 mg). Again, evaporation of the sub-fractions 74-76 yielded to the isolation of whitish mass which was purified by repeated preparative TLC using toluene-ethyl acetate (90:10) to afford compound **7** (7.10 mg). Similarly, a portion of the chloroform soluble fraction (500 mg) was subjected to column chromatography using petroleum ether followed by petroleum ether-ethyl acetate and ethyl acetate-methanol gradients to obtain 170 sub-fractions 20 ml each. Evaporation of solvents from sub-fractions 59-69 yielded a yellowish mass. This yellowish mass was subjected to preparative TLC using chloroform-methanol (97:3) to give compound **8** (3.54 mg).

Properties of isolated compounds. Glochidonol (**1**): white crystals; mp 227-229 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.66 (2H, d, *J*= 1.6 Hz, H_b-29), 4.54 (2H, s, H_a-29), 3.80 (1H, d, *J*= 2.8 Hz, H-1), 2.99 (2H, dd, *J*= 14.3, 8.1 Hz, H_a-2), 2.38 (2H, m, H_b-2), 1.66 (3H, s, Me-30), 1.03 (3H, s, Me-23 & Me-24), 1.01 (3H, s, Me-26), 0.95 (3H, s, Me-27), 0.80 (3H, s, Me-25), 0.78 (3H, s, Me-28). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 79.0 (C-1), 45.3 (C-2), 215.4 (C-3), 47.2 (C-4), 53.3 (C-5), 18.9 (C-6), 33.0 (C-7), 43.0 (C-8), 49.4 (C-9), 43.0 (C-10), 22.5 (C-11), 25.7 (C-12), 38.1 (C-13), 41.5 (C-14), 27.6 (C-15), 35.6 (C-16), 43.1 (C-17), 48.4 (C-18), 48.0 (C-19), 150.8 (C-20), 28.4 (C-21), 39.9 (C-22), 27.9 (C-23), 20.5 (C-24), 11.8 (C-25), 16.4 (C-26); 14.9 (C-27), 18.2 (C-28), 109.7 (C-29), 20.4 (C-30).

Brassicasterol (**2**): colorless needles; mp 153-156 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.36 (1H, d, *J* = 6.0, H-6), 5.22 (1H, m, H-23), 5.17 (1H, m, H-22), 3.62 (1H, m, H-3), 1.21 (3H, d, *J*= 6.0, Me-28), 1.00 (3H, d, *J*= 6.5, Me-21), 0.93 (3H, d, *J*= 6.5, Me-27), 0.84 (3H, d, *J*= 6.5, Me-26), 0.83 ((3H, s, Me-19), 0.69 ((3H, s, Me-18).

Friedelin (**3**): needles; mp 268 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.19 (3H, s, Me-28), 1.06 (3H, s, Me-27), 1.02 (3H, s, Me-26), 1.01 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.90 (3H, d, *J*= 6.4 Hz, Me-23), 0.88 (3H, s, Me-25), 0.74 (3H, s, Me-24). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 22.5 (C-1), 41.5 (C-2), 213.5 (C-3), 58.5 (C-4), 42.4 (C-5), 41.8 (C-6), 18.5 (C-7), 53.3 (C-8), 37.7 (C-9), 59.7 (C-10), 35.9 (C-11), 30.1 (C-12), 39.2 (C-13), 38.5 (C-14), 32.9 (C-15), 36.0 (C-16), 30.2 (C-17), 43.0 (C-18), 35.4 (C-19), 28.4 (C-20), 32.6 (C-21), 39.5 (C-22), 7.0 (C-23), 14.8 (C-24), 18.1 (C-25), 20.4 (C-26), 18.9 (C-27), 32.0 (C-28), 35.0 (C-29), 31.3 (C-30).

5*β*-24S-Ethylcholestane-3*β*-ol (**4**): whitish mass; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.99 (1H, m, H-3), 0.93 (3H, s, Me-19), 0.92 (3H, d, *J*= 6.4 Hz, Me-21), 0.89 (3H, t, *J*= 7.5 Hz, Me-29), 0.87 (3H, d, *J* = 8.0 Hz, Me-26), 0.83 (3H, d, *J* = 7.2 Hz, Me-27), 0.70 (3H, s, Me-18).

Stigmasterol (**5**): white crystals; mp 163°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.35 (1H, m, H-6), 5.14 (1H, dd, *J*= 15.0 and 6.5 Hz, H-22), 5.04 (1H, dd, *J*= 15.0 and 6.5 Hz, H-23), 3.49 (1H, m, H-3), 1.00 (3H, s, Me-19), 0.91 (3H, d, *J*= 6.2 Hz, Me-21), 0.90 (3H, d, *J*= 6.5 Hz, Me-20), 0.82 (3H, t, *J*= 6.5 Hz, Me-28), 0.67 (3H, s, Me-18).

3-Ketooleanane (**6**): white crystals; mp 235-237 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.25 (3H, s, Me-23), 1.18 (3H, s, Me-24), 1.05 (3H, s, Me-27), 1.01 (3H, s, Me-29), 1.00 (3H, s, Me-30), 0.96 (3H, s, Me-28), 0.87 (3H, s, Me-25), 0.72 (3H, s, Me-26).

5-5'-Dihydroxysesamine (**7**): colorless semisolid; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.95 (1H, d, *J*= 1.6 Hz, H-6'), 6.92 (1H, d, *J*= 1.6 Hz, H-2'), 6.88 (1H, dd, 1.6, 0.8 Hz, H-6"), 6.86 (1H, bs, H-2"), 6.80 (1H, d, 7.6 Hz, H-5"), 5.99 (s, -OCH_2O-), 5.96 (s, -OCH_2O-), 4.84 (1H, d, 5.2 Hz, H-2β, H-6β), 4.52 (2H, dd, 9.2, 8.0 Hz, H-8ax), 4.05 (2H, d, 9.6 Hz, H-4eq), 3.92 (2H, d, 9.6 Hz, H-4ax), 3.85 (2H, dd, 9.0, 8.0 Hz, H-8eq), 3.01 (1H, m, H-1).

Pinoresinol (**8**): yellowish mass; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.88 (1H, d, 8.0 Hz, H-5', H-5"), 6.86 (1H, d, 1.6 Hz, H-2', H-2"), 6.81 (1H, dd, 8.0, 1.6 Hz, H-6', H-6"), 5.56 (1H, s, H-4', H-4"), 4.72 (1H, d, 4.0 Hz, H-2, H-6), 4.17 (2H, dd, 9.2, 7.2 Hz, H-4eq, H-8eq), 3.84 (s, OMe-3', 3"), 3.81 (2H, dd, 8.8, 3.6 Hz, H-4ax, H-8ax), 3.09 (1H, m, H-1, H-5).

Biological studies. The cytotoxic, antimicrobial and free radical scavenging activities of the isolated compounds were determined by the established methods.[8-13]

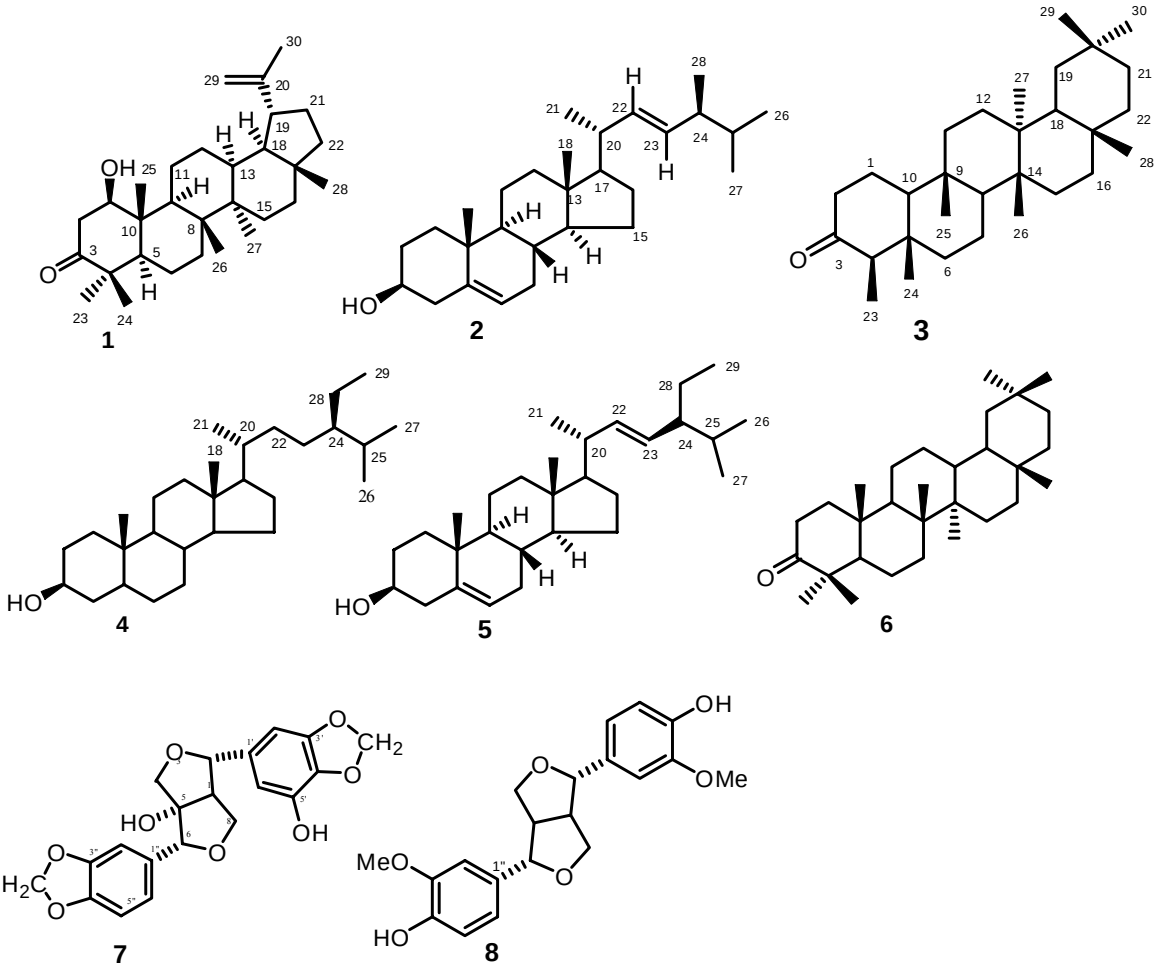
RESULTS AND DISCUSSION

A total of eight compounds (**1-8**) were isolated from the methanol extract of stem bark of *B. verrucosa* by column chromatography, PTLC and recrystallization. The structure of the isolated compounds were elucidated by high field NMR analysis and comparison with the published values.

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **1** exhibited typical signals for 48 protons including seven tertiary methyl, a carbinol and a terminal exomethylene protons. It also showed seven singlets with three proton intensity indicating seven tertiary methyl groups at δ 1.03 (H-23), 1.03 (H-24), 0.80 (H-25), 1.01 (H-26), 0.95 (H-27), 0.78 (H-28) and 1.66 (H-30). The spectrum also demonstrated a one proton broad doublet at δ 3.80 (1H, d, *J*= 2.8 Hz) corresponding to oxymethine proton at C-1. It also showed olefinic protons at δ 4.54 (H_a-29) and δ 4.66 (H_b-29, d, *J*= 1.6 Hz). The ^{13}C-NMR displayed a total of 30 carbon resonances including a carbonyl carbon at δ 215.4 and also showed typical signal at δ 79.0 for the oxymethine carbon at C-1 while two signals at δ 150.8 and δ 109.7 could be assigned to C-20 and C-29, respectively. All these features are indicative of the presence of a lupane-type triterpenoid type skeleton in compound **1**. The melting point and ^{1}H and ^{13}C-NMR spectral data of compound **1** were compared with the published values of glochidonol and found to be identical.[14] Thus, compound **1** was characterized as glochidonol which is the first report from *B. verrucosa*.

The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of compound **2** displayed a multiplet of one proton at δ 3.62 for the oxymethine proton at H-3. The typical signal for the olefinic C-6 proton of the steroidal skeleton was evident from a doublet at δ 5.36 (*J*= 6.0

Hz) that integrated for one proton. The *trans*-olefinic protons H-22 and H-23 appeared as characteristic downfield signals at δ 5.17 (1H, m) and δ 5.20 (1H, m) due to couplings with the neighbouring olefinic and methine protons. The spectrum further revealed signals at δ 0.69 and 0.83 (3H each) assignable to two tertiary methyl groups at C-13 (H_3-18) and C-10 (H_3-19), respectively. Four doublets of three proton intensity at δ 1.00 (3H, d, *J* = 6.5 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.93 (3H, d, *J* = 6.5 Hz) and1.21 (3H, d, *J* = 6.5 Hz) were assigned to the methyl groups at Me-21, Me-26, Me-27, Me-28, respectively. All these features are indicative of the presence of a steroidal nucleus. Furthermore, the melting point and ^{1}H-NMR spectral data compound **2** were compared with the reported values of brassicasterol and found to be identical.[15] Thus, compound **2** was characterized as brassicasterol which is also the first report from *B. verrucosa*.



In case of compound **3,** the ^{1}H-NMR spectrum showed seven singlets between δ 0.74-1.19 corresponding to seven methyl groups and a doublet of three proton intensity at δ 0.90 (3H, *J*= 6.4 Hz). These confirmed the presence of eight methyl groups in a pentacyclic type triterpenoid skeleton. However, the lack of any olefinic proton resonance around δ 5.00-5.50 and oxymethine proton signal around δ 3.00 demonstrated the absence of double bond between C-12 and C-13 and the typical oxymethine proton at C-3. The ^{13}C-NMR displayed 30 carbon resonances including a carbonyl carbon at δ 213.54

ppm. The DEPT spectrum indicated that twenty three out of the thirty carbons in compound **3** had attached protons. The DEPT experiment further suggested the presence of eight methyl, eleven methylene, four methine and seven quarternary carbon atoms. All these features are indicative of the presence of a triterpenoid type carbon skeleton in compound **3** having no double bond between C-12 and C-13, and no oxymethine proton at C-3. The melting point, ^{1}H and ^{13}C-NMR spectral data of compound **3** were compared with the published data of friedelin and found to be identical.[16] Thus, compound **3** was characterized as friedelin which is also the first report from *B. verrucosa*.

The ^{1}H-NMR spectrum of compound **4** revealed a one proton multiplet at δ 3.75 which was indicative of H-3 in a steroidal nucleus. It also displayed signals of two proton signals at δ 0.93 and 0.70 indicating two tertiary methyl groups at C-10 and C-13. The doublets (each 3H intensity) at δ 0.87 (d, *J*= 8.0 Hz) and 0.83 (d, *J*= 7.2 Hz) were observed due to the secondary methyl groups at C-25. Another doublet at δ 0.92 (d, *J*= 6.4 Hz) was assigned to a methyl group at C-20. A triplet of three proton intensity at δ 0.89 (*J*= 7.5 Hz) could be attributed to primary methyl group at C-28. The absence of any signals between δ 5.00 and 6.00 suggested the absence of olefinic proton between C-5 and C-6 as usually observe in sitosterol and stigmasterol type of steroidal compounds. These spectral features were found to be in close agreement with those of 5*β*-24S-ethylcholestan-3*β*-ol[17] which is the first report from *B. verrucosa*.

The ^{1}H-NMR spectrum of compound **5** revealed a one proton multiplet at δ 3.49 which was indicative of H-3 in a steroidal nucleus. The typical C-6 proton was evident as a broad singlet at δ 5.40 that integrated for one proton. The olefinic protons H-22 and H-23 appeared as characteristic downfield signals at δ 5.14 (dd, *J*= 15.0, 6.5 Hz) and δ 5.04 (dd, *J*= 15.0, 6.5 Hz) which were comparable to the ^{1}H-NMR data of authentic stigmasterol. It further displayed signals of three proton intensity at δ 0.67 and 1.00 indicating the presence of two tertiary methyl protons at C-10 and C-13. The remaining doublets (each 3H) at δ 0.80 and 0.84 (*J*= 6.0 Hz) were assigned to the secondary methyl groups at C-25 and a triplet at δ 0.82 (*J*= 6.5 Hz) was due to primary methyl group at C-29. Another doublet near δ 0.90 (*J*= 6.5 Hz) was assigned to a methyl group at C-20. These spectral features as well as the melting point of compound **5** were found to be in close agreement with those of stigmasterol.[18] Moreover, the spectrum of compound **5** was superimposable to the ^{1}H-NMR spectrum acquired for an authentic stigmasterol previously isolated in our laboratory. So, compound **5** was confirmed as stigmasterol.

The ^{1}H-NMR spectrum of compound **6** exhibited eight singlets corresponding to eight methyl groups between δ 0.72-1.25. However, the absence of any olefinic proton resonance near δ 5.00 and oxymethine proton signal around 3.00 suggested the absence of double bond between C-12 and C-13, and the oxymethine proton at C-3. All these are indicative of the presence of a pentacyclic tyre triterpenoid skeleton in compound **6** having no double bond between C-12 and C-13. The melting point and the ^{1}H-NMR spectral data were compared with the published data of 3-ketooleanane and found to be identical.[19] Thus, compound **6** was characterized as 3-ketooleanane which is the first report of its isolation from *B. verrucosa*.

The ^{1}H-NMR spectral data of compound **7** could be assigned by careful comparison of the data reported for structurally related compound, sesamine[20,21] as well as with the help of ^{1}H-^{1}H COSY spectrum. It showed a one proton multiplet at δ 3.05 which could be assigned to the methine proton, H-1. In the ^{1}H-^{1}H COSY spectrum it was found to couple with a vicinal proton at δ 4.84 (d, *J*= 5.2 Hz, H-2) and also with a pair of methylene protons at δ 3.85 (dd, *J*= 9.0 and 6.0 Hz, H-8eq) and δ 4.52 (dd, *J*= 9.0 and 6.0 Hz, H-8ax). Another pair of methylene protons were observed at δ 4.05 (d, *J*= 9.6 Hz, H-4eq) and δ 3.92 (d, *J*= 9.6 Hz, H-4ax) which were geminally coupled to each other only indicating the absence of a vicinal proton at H-5, where a hydroxyl group may be present in compound

7 as compared to a hydrogen at C-5 in sesamine. Jayasinghe *et al*., (2003) reported the presence of an aromatic proton at H-5' in sesamine which was coupled with the vicinal aromatic proton H-6' to give a doublet with a *J* value of 7.90 Hz, a typical ortho coupling but H-6' proton in compound **7** it appeared as a doublet (*J*= 1.6 Hz) at δ 6.90 Hz due to the presence of H-2' aromatic proton indicating the absence of H-5' proton.[21] So, there may be a OH group in compound **7**. From the above spectral evidences it can be assumed that the isolated compound **7** could be an asymmetrically substituted 2,6-diaryl *cis*-3,7-dioxabicyclo [3.3.0] octane derivative.[20] On the basis of the above spectral data as well as by comparison with that of sesamine, the proton chemical shifts of compound **7** have been assigned. The remaining spectral data were similar to that of the reported compound sesamine. So compound **7** was tentatively identified as 5,5'-dihydroxysesamine. This is the first report of occurrence of 5,5'-dihydroxysesamine from any natural and synthetic sources. However, additional spectral data is required to confirm its structure.

The ^{1}H-NMR spectrum of compound **8** indicated the presence of typical pattern of two 1,3,4-trisubstituted benzene rings with signals at δ 6.88 (2H, d, *J*= 8.0 Hz, H-5', H-5"), δ 6.81 (2H, dd, *J*= 8.0, 1.6, H-6', H-6") and δ 6.86 (2H, d, *J*= 1.6 Hz, H-2', H-2") and two methoxy groups at δ 3.84 (6H). It also revealed a multiplet of two proton intensity at δ 3.09 for H-1 and H-5 and a doublet for H-2 and H-6 at δ 4.72 with *J* value of 4.00 Hz. Another double doublet at δ 4.17 (*J*= 9.2, 7.2 Hz) that integrated for two protons, could be attributed to H-4_{eq} and H-8_{eq}.[22] The ^{1}H-NMR spectrum further revealed showed a double doublet at δ 3.81 (*J*= 8.8 and 3.6) for two protons assignable to H-4_{ax} and H-8_{ax}. These ^{1}H-NMR spectral data indicated that compound 8 was a lignan of the 2,6-diaryl-3,7-dioxobicyclo [3.3.0] octane type.[20] Moreover, all the other proton shifts were similar to that published for pinoresinol.[23] So, compound **8** was identified as pinoresinol which also the first report from *B. verrucosa*.

In case of brine shrimp lethality bioassay the LC_{50} and LC_{90} were obtained from the best-fit slope when the data was presented graphically for the compounds **3, 7** and **8** isolated from *B. verrucosa*. Compound **7** revealed promising LC_{50} and LC_{90} values of 8.11 and 109.14 µg/ml, respectively. On the other hand, compound **3** and **8** exhibited 13.80 and 10.12 µg/ml, respectively for LC_{50}, and 213.79 and 164.82 µg/ml, respectively for LC_{90}, when compared to the standard vincristine sulphate (LC_{50} value 0.45 µg/ml and LC_{90} value of 10.00 µg/ml).

Table 1. Results of the brine shrimp lethality bioassay of the compounds 3, 7 and 8.

Compound	LC_{50} µg/ml	LC_{90} µg/ml
3	13.80	213.79
7	8.11	109.14
8	10.12	164.82

Compounds **3**, **7** and **8** when subjected to antimicrobial screening showed weak to strong activity with zones of inhibition ranging from 11 to17 mm at a concentration of 50 µg/disc against various Gram positive and Gram negative bacterial strains by comparison with standard kanamycin discs (Table 2). The highest zone of inhibition (16.7 mm) was produced by compound **8** against *S. dysenteriae*.

In case of antifungal screening, the zone of inhibition was observed between 10 and 16 mm indicating low to mild activity in contrast to griseofulvin as standard. However, compound **8** showed highest activity against *A. niger* with zone of inhibition of 15.3 mm.

The free radical scavenging activity of compound **7** was found to be highest having IC_{50} value of 7.3 µg/ml and the lowest activity was shown by the compound **3** with IC_{50} being 17.9 µg/ml. The remaining two compounds **4** and **8** demonstrated high to moderate free radical scavenging activity with IC_{50} value being 14.7 and 8.4 µg/ml, respectively as compared to the standards, i.e. tert-butyl-1-hydroxytoluene (BHT), (IC_{50} = 24.35 µg/ml) and ascorbic acid, ASA (IC_{50} = 5.80 µg/ml).

Table 2. Antimicrobial activity of compounds 3, 7 and 8 isolated from *B. verrucosa*

Test bacteria and fungi	Diameter of zone of inhibition (mm)			
	Compound **3** (50 µg/disc)	Compound **7** (50 µg/disc)	Compound **8** (50 µg/disc)	Kanamycin (30 µg/disc)
Gram positive bacteria				
Bacillus cereus	11.9 ± 0.33	13.9 ± 0.75	13.5 ± 0.71	35
B. megaterium	13.1 ± 0.31	14.5 ± 0.66	14.6 ± 0.41	35
B. subtilis	12.1 ± 0.54	12.8 ± 0.46	12.3 ± 0.38	36
Staphylococcus aereus	12.9 ± 0.91	12.2 ± 0.29	13.0 ± 0.78	32
Sarcina lutea	14.6 ± 0.55	14.5 ± 0.57	11.8 ± 0.66	27
Gram negative bacteria				
Escherichia coli	13.2 ± 0.58	12.1 ± 0.49	12.9 ± 0.71	25
Pseudomonas aeruginosa	13.1 ± 0.62	13.5 ± 0.31	14.6 ± 0.41	20
Salmonella paratyphi	14.8 ± 0.31	13.1 ± 0.74	14.3 ± 0.55	27
S. typhi	12.8 ± 0.31	15.1 ± 0.76	16.5 ± 0.46	22
Shigella boydii	15.2 ± 0.76	16.4 ± 0.36	14.6 ± 0.46	27
S. dysenteriae	14.3 ± 0.55	14.8 ± 0.76	16.7 ± 0.35	25
Vibrio mimicus	13.3 ± 0.91	14.2 ± 0.31	13.5 ± 0.35	25
V. parahemolyticus	13.1 ± 0.66	13.9 ± 0.74	14.6 ± 0.22	20
Fungi				**Griseofulvin (20 µg/disc)**
Aspergillus niger	13.7 ± 0.51	14.4 ± 0.36	15.3 ± 0.31	20
Candida albicans	14.3 ± 0.31	13.7 ± 0.45	10.4 ± 0.55	18
Saccharomyces cerevisiae	14.7 ± 0.47	13.7 ± 0.45	13.1 ± 0.67	19

Table 3. Free radical scavenging activity (IC_{50} in µg/ml) of the compounds 3,4,7 and 8.

Compound	IC_{50} (µg/ml)
3	17.9
4	14.7
7	7.3
8	8.4

CONCLUSION

Fractionation and purification of the stem bark of *B. verrucosa* collected from Khagrachhori, Chittagong, Bangladesh afforded eight compounds, which were identified as glochidonol (**1**), brassicasterol (**2**), friedelin (**3**), 5*β*-24S-ethylchoestane-3*β*-ol (**4**), stigmasterol (**5**), ketooleanane (**6**), 5-5'-dihydroxy sesamine (**7**) and pinoresinol (**8**). Among these compound **7** appears to be new, while compounds **1**, **2**, **3**, **4**, **6** and **8** are the first report of their isolation from *B. verrucosa*. Though compound **7** was first isolated from natural source but additional spectral data (MS and ^{13}C NMR data) is required to confirm its structure. Compound **7** showed promising cytotoxic activity against brine shrimp nauplii. Compounds **3** and **8** revealed mild to moderate antibacterial activity against a number of Gram positive and Gram negative bacteria when compaired to kanamycin. The compounds also showed satisfactory antifungal activity against a number of fungal strains. The free radical scavenging activity of compound **7** was also promising. Since the plant *B. verrucosa* has a number of bioactive compounds, it might be a source of drug for various diseases.

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