# Bioactive Steroid and Triterpenoids from *Bridelia stipularis* (L) Blume

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**ABSTRACT:** Fractionation and purification of stem bark extract of *Bridelia stipularis* growing in Bangladesh afforded glut-5(6)-en-3-one (**1**), glut-5(6)-en-3α-ol (**2**), and (22*E*)-7-hydroxy-28-methylcholesta-4,22-dien-3-one (**3**). Compound **3** appears to be new, while compounds **1** and **2** have never been reported from this plant. The isolated compounds isolated exhibited cytotoxic activity against brine shrimp nauplii having significant $LC_{50}$ and $LC_{90}$ and moderate to strong antimicrobial activity against 13 Gram positive and Gram negative bacterial strains and 3 fungi. Here, compound **1** demonstrated highest inhibition of growth of microorganisms with zone of inhibition of 22.7 mm against *Escherichia coli* and compound **2** displayed zone of inhibition of 20.8 mm against *Candida albicans*. Compounds **1-2** also revealed moderate free radical scavenging activity in the DPPH method.

**Key words:** *Bridelia stipularis*, triterpenes, steroids, antimicrobial, cytotoxity, free radical scavenging

## INTRODUCTION

*Bridelia stipularis* (L) Blume (Synonym: *Clutia stipularis* L., *B. scandens*, Local Bengali name: Pat Khowi, Family: Phyllanthaceae) is a climbing shrub, which grows in shady, moist forest floors. It is distributed in the forest areas of the central and eastern parts of Bangladesh.[1] It is also found in India and Myanmar. The plant is used in the treatment of amoebic dysentery, chest pain, constipation, diarrhea, leucoderma and strangury.[2] Decoction of bark is used for cough, fever and asthma. It has also shown hypotensive and hypoglycaemic actions in animal model, where the leaves are used for jaundice.[3] As part of our continuing studies on *Bridelia* species growing in Bangladesh[4,5] we attempted to isolate and identify the chemical constituents from the stem bark of *B. stipularis* and assayed for the biological activities of the isolated compounds.

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## MATERIALS AND METHODS

**General experimental procedures.** Column chromatography was carried out on 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 (PTLC) were run on precoated silica gel plates Kieselgel 60 $F_{254}$, Aluminum sheets, E-Merck, Germany). Melting points were measured on a hot stage melting point apparatus (PIC, India) and are uncorrected. $^1$H NMR spectra were recorded on a Bruker 400 MHz spectrometer and the chemical shifts are expressed in ppm with respect to residual non-deuterated solvent signal.

**Plant materials.** The stem bark of *Bridelia stipularis* (L) Blume was collected from the village of Panchouri, Khagrachhori District in February, 2007 and identified in Bangladesh National Herbarium, where voucher specimen has been deposited representing this collection (Accession No. DACB- 31378).

**Extraction and isolation.** The air dried powdered stem bark of *B. stipularis* (550 g) was soaked in 1.5 L methanol for seven days and filtered through fresh cotton bed followed by Whatman number 1 filter paper. The filtrate was concentrated by using a rotary evaporator at 40 ºC. A portion of the methanolic crude extract (15 g) was subjected to VLC over silica gel (column length 14 cm and diameter 10 cm which was packed up to a height of 6 cm under reduced pressure). After loading the sample the column was eluted with petroleum ether, followed by mixtures of petroleum ether and ethyl acetate and finally with methanol to obtain 25 fractions of 100 ml each. After TLC screening similar fractions (4 to 5A) were combined together and concentrated under reduced pressure. They were then subjected to preparative TLC using *n*-hexane-ethyl acetate (99:1) to yield compound **1** (8.5 mg). Fractions 9A to 11B were also combined together and subjected to gel permeation chromatography over Sephadex LH-20, that eluted with *n*-hexane-dichloromethane-methanol (2:5:1). Fifty five fractions were obtained and fractions with similar TLC feature were combined. Preparative TLC of fractions 20-26 over silica gel using toluene-ethyl acetate (97:3) provided compounds **2** (5.5 mg) and **3** (7.0 mg).

**Properties of isolated compounds. Glut-5(6)-en-3-one (1):** white crystals; mp 207-210 ºC; $^1$H NMR (400 MHz, $CDCl_3$): δ 5.61 (1H, m, H-6), 1.17 (3H, s, Me-24), 1.16 (3H, s, Me-23), 1.10 (3H, s, Me-28), 1.03 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.93 (3H, s, Me-30), 0.89 (3H, s, Me-29), 0.75 (3H, s, Me-25).

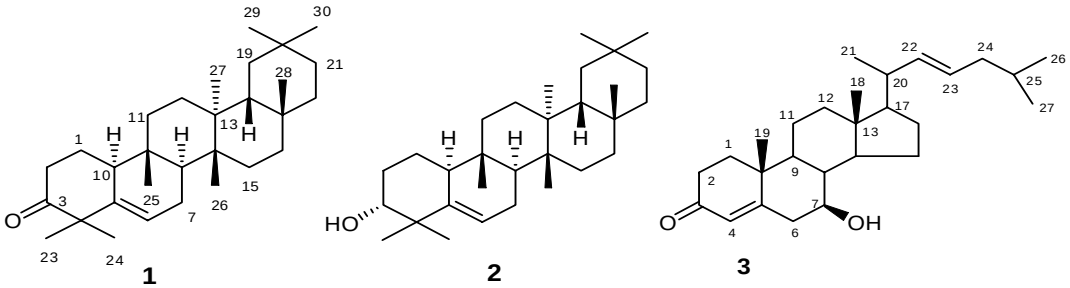
**Glut-5(6)-en-3α-ol (2):** white needles; mp 203 ºC; $^1$H NMR (400 MHz, $CDCl_3$): δ 5.64 (1H, d, *J*= 6.4 Hz, H-6), 3.47 (1H, t, *J*= 2.8 Hz, H-3), 1.17 (3H, s, Me-23), 1.14 (3H, s, Me-28), 1.10 (3H, s, Me-29), 1.05 (3H, s, Me-27), 1.01 (3H, s, Me-24), 0.99 (3H, s, Me-26), 0.95 (3H, s, Me-30), 0.85 (3H, s, Me-25).

**(22*E*)-7-Hydroxy-28-methylcholesta-4,22-dien-3-one (3):** white amorphous powder; $^1$H NMR (400 MHz, $CDCl_3$): δ 5.70 (1H, bs, H-4), 5.20 (1H, dd, *J*= 15.0, 8.5 Hz, H-23), 5.06 (1H, dd, *J*= 15.5, 9.0 Hz, H-22), 3.67 (1H, m, H-7), 1.33 (3H, s, Me-19), 1.27 (3H, d, *J*= 6.7 Hz, Me-21), 0.85 (3H, d, *J*= 6.8 Hz, Me-26 and Me-27), 0.84 (3H,s, Me-28), 0.70 (3H, s, Me-18).

**Biological studies.** The cytotoxic, antimicrobial and free radical scavenging activities of the isolated compounds (**1** and **2**) were determined by following the established methods.[6-11]

## RESULTS AND DISCUSSION

A total of 3 compounds (**1-3**) were isolated from a methanol extract of stem bark of *B. stipularis* by column chromatography, gel permeation chromatography and preparative TLC. The structures of the isolated compounds were primarily solved by high field NMR data analysis and comparison with published values.




**1** **2** **3**

Compound **1** was characterized as glut-5(6)-en-3-one by careful analysis of its high resolution $^1$H NMR data comparison with reported values[12] and further confirmed by melting point as well as co-TLC with an authentic glut-5(6)-en-3-one previously isolated in our laboratory. Therefore, the compound **1** was characterized as glut-5(6)-en-3-one, which is the first report from *B. stipularis*.

Compound **2** melted at 203 $^{o}$C, which was identical to that reported for glut-5(6)-en-3-one.[12] The $^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **2** displayed an olefinic proton signal at δ 5.64 (1H, d, *J*= 6.4 Hz) and an oxymethine proton at 3.47 (1H, t, *J*= 2.8 Hz), which were assigned to H-6 and H-3, respectively of a triterpenoid type carbon skeleton. The oxymethine proton at δ 3.47 was axial in configuration as confirmed by a triplet with *J* value 2.8 Hz.[13] The $^{1}$H NMR spectrum also showed eight methyl group resonances as singlets at δ 0.85, 0.95, 0.99, 1.01, 1.05, 1.10, 1.14 and 1.17. On the basis of the above spectral data, compound **2** was characterized as glut-5(6)-en-3α-ol.[12] The identity of compound **2** as glut-5(6)-en-3α-ol was substantiated by comparison of its spectral data with published values as well as by co-TLC with authentic sample. This is the first report of isolation of glut-5(6)-en-3α-ol (**2**) from *B. stipularis*.

**Table 1. Antimicrobial activity of compounds 1 and 2 at 50 µg/disc isolated from *B. stipularis***

| Test bacteria and fungi | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|
| | Compound 1 (50 µg/disc) | Compound 2 (50 µg/disc) | Kanamycin (30 µg/disc) |
| **Gram positive bacteria** | | | |
| *Bacillus cereus* | 19.0 ± 0.60 | 16.1 ± 0.66 | 35 |
| *B. megaterium* | 18.4 ± 0.50 | 16.9 ± 0.91 | 35 |
| *B. subtilis* | 17.5 ± 0.87 | 16.3 ± 1.05 | 36 |
| *Staphylococcus aereus* | 17.8 ± 0.40 | 18.2 ± 0.89 | 32 |
| *Sarcina lutea* | 21.6 ± 0.87 | 20.7 ± 1.22 | 27 |
| **Gram negative bacteria** | | | |
| *Escherichia coli* | 22.7 ± 0.65 | 18.8 ± 0.42 | 25 |
| *Pseudomonas aeruginosa* | 17.9 ± 0.76 | 18.4 ± 0.76 | 20 |
| *Salmonella paratyphi* | 20.1 ± 0.65 | 18.4 ± 0.76 | 27 |
| *S. typhi* | 22.1 ± 0.65 | 20.6 ± 0.86 | 22 |
| *Shigella boydii* | 20.2 ± 1.29 | 18.1 ± 0.86 | 27 |
| *S. dysenteriae* | 21.2 ± 0.36 | 19.2 ± 0.66 | 25 |
| *Vibrio mimicus* | 21.3 ± 0.91 | 16.9 ± 0.61 | 25 |
| *V. parahemolyticus* | 20.7 ± 0.60 | 18.9 ± 0.59 | 20 |
| **Fungi** | | | Griseofulvin (20 µg/disc) |
| *Aspergillus niger* | 19.1 ± 0.35 | 18.2 ± 0.59 | 20 |
| *Candida albicans* | 18.5 ± 0.60 | 20.8 ± 0.45 | 18 |
| *Saccharomyces cerevisiae* | 18.2 ± 0.31 | 20.2 ± 0.83 | 19 |

The $^{1}$H NMR spectrum of the compound **3** exhibited resonances at δ 0.70 (3H, s), 0.85 (6H, d, *J*= 6.8 Hz, overlapped), 1.27 (3H, d, *J*= 6.7 Hz) and 1.30 (3H, s) attributable to the methyl groups at C-18, C-26/27, C-21 and C-19, respectively in a cholestane type carbon skeleton.[14] In the $^{1}$H NMR spectrum, the olefinic protons at H-22 and H-23 appeared as characteristic downfield signals at δ 5.20 (1H, m) and 5.06 (1H, m).[14] Another olefinic proton signal at δ 5.70 (1H, bs) was attributed to the trisubstituted double bond conjugated with the ketone group located at C-3, and therefore, the trisubstituted double bond could be placed between at C-4 and C-5.[15] The $^{1}$H-NMR spectrum further revealed signals for an oxymethine proton at δ 3.67 (1H, m) and a tertiary methyl proton at δ 0.84 indicating a hydroxyl group at C-7 and a methyl group at C-28, respectively.[16] In the literature, it was revealed that if the hydroxyl

group was situated to C-24, the proton chemical shifts of C-24, C-22 and C-23 could move upfield to δ 3.77, 5.49 and 5.38, respectively which do not match with the resonances observed in the $^1$H NMR spectrum of compound **3**. On this basis as well as by comparison of $^1$H NMR data with two steroidal ketones reported in the literatures,[14,15] compound **3** was tentatively characterized as (22*E*)-7-hydroxy-28-methylcholesta-4,22-dien-3-one. This is the first report of occurrence of compound **3** from any natural and synthetic sources. However, additional spectral data are required to confirm its structure.

**Biological studies.** In case of cytotoxicity screening by brine shrimp lethality bioassay, the $LC_{50}$ and $LC_{90}$ values of the isolated compounds **1** and **2** were obtained from the best-fit slope when the data was presented graphically. Compound **1** revealed promising $LC_{50}$ and $LC_{90}$ values of 7.64 and 101.16 µg/ml, respectively. On the other hand, compound **2** exhibited 8.53 µg/ml for $LC_{50}$ and 218.27 µg/ml for $LC_{90}$, when compared to the standard vincristine sulfate ($LC_{50}$ value 0.45 µg/ml).

Compounds **1** and **2** were subjected to antimicrobial screening which also demonstrated moderate to strong activity with zones of inhibition ranging from 10 to 21 mm at a concentration of 50 µg/disc against different Gram positive and Gram negative bacteria and fungal strains in comparison to standard kanamycin and griseofulvin, respectively (Table 1). The highest zone of inhibition (22.7 mm) was produced by compound **1** against *E. coli*. Compound **2** showed highest activity against *C. albicans* with zone size of 20.8 mm.

In addition, compound **2** showed moderate free radical scavenging activity with the $IC_{50}$ value being 12.6 µg/ml 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).

It has been reported that structurally related triterpenes and steroids showed moderate to strong cytotoxic, antimicrobial and free radical scavenging activities.[16-18] Therefore, the bioactivities of compounds **1**-**3** are in accordance with the previous findings.

## CONCLUSION

Fractionation and purification of the methanolic extract of stem bark of *B. stipularis* collected from Panchouri, District Khagrachhori, Bangladesh afforded three compounds which were identified as glut-5(6)-en-3-one (**1**), glut-5(6)-en-3α-ol (**2**), and (22*E*)-7-hydroxy-28-methylcholesta-4,22-dien-3-one (**3**). Compound **3** appears to be new, and compounds **1** and **2** are the first report of their isolation from *B. stipularis*. Like other triterpenoids, compound **1** showed promising cytotoxic activity and compounds **1** and **2** demonstrated moderate to strong antibacterial activity against some Gram positive and Gram negative bacteria. They also showed good antifungal activity against a number of fungal strains.

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