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Triterpenoids and phytosteroids from stem bark of *Crataeva nurvala* buch ham.

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ABSTRACT

Crude rectified spirit extract of the stem bark of *Crataeva nurvala* was taken under chemical investigation. The chloroform fraction yielded total four compounds namely betulinic acid (1), lupeol (2) and β -sitosterol (3) and stigmasterol (4). The structure of the isolated compounds were established by extensive analyses of ^1H NMR spectroscopy and compared the spectral data, melting points with the authentic specimen. The results indicate that the stem bark of *Crataeva nurvala* provide a rich source of triterpenoids and steroids.

Keywords: Capparidaceae, *Crataeva nurvala*, triterpenoids, stigmasterol, betulinic acid, lupeol, β -sitosterol.

INTRODUCTION

The discovery of a novel chemical component from a medicinal plant may form the basis of development of various therapeutic agents with better activity. More than 500 medicinal plants have been reported to possess medicinal properties in Bangladesh and *Crataeva nurvala* Buch Ham, (family: Capparidaceae) is one of the most common species among them. The plant *Crataeva nurvala* Buch Ham belonging to the family Capparidaceae is a leafy small to medium sized soft wooded tree with fragrant white flowers and oblong to rounded hard fruits, grows on the banks of canals, rivers, lakes etc (Kirtikar & Basu, 2005). The evergreen tree grows widely in all parts of Bangladesh, Pakistan, India, Philippine, South America, China, and Africa. The common names are Borun or Bonna Pithagola (Bengali), Barna (Hindi), Varuna (Sanskrit), three leaved caper (English) (Ghani, 2003). The bark of the tree is an important drug for problem affecting the kidneys and bladder. It is especially effective in the urinary complaints, kidney and bladder stones (inhibit the formation of stones), fever, vomiting and gastric irritation (Ghani, 2003). It also acts as contraceptive and oxytocic; juice of bark is given to women after childbirth. Root and bark are also laxative and lithontripic. They increase appetite and biliary secretion (Malini et al., 1995). Leaves are externally rubefacient and used in rheumatism; internally they are given as febrifuge and tonic. Phytochemical studies showed that stem bark of the plant contains saponins, flavonoids, sterols and glucosilnates and ceryl alcohol, friedelin, cadabicine diacetate, lupeol, betulinic acid and diosgenin (Lakshmi & Chauhan, 1975). Fruits contain glucocapparin, beta-sitosterol, triacontane, triacontanol, cetyl and ceryl alcohol. Leaves contain L-stachydrine, dodecanoic anhydride, methyl pentacosanoate, kaemferol-0- α -D-glucoside and quercitin-3-0- α -D-glucoside (Gagandeep & Kalidhar, 2006). Root bark contains rutin, quercitin, lupeol, varunol and β -sitosterol. Presence of alkaloids has been reported in bark and stems (Ghani, 2003).

We herein report the chemical constituents present in the stem bark extract by chromatographic and spectrophotometric analysis and established the presence of triterpenoids as well as steroids.

MATERIALS AND METHODS

General experimental procedure

Melting points were determined on a Stuart hot stage melting point apparatus and are uncorrected. The ^1H spectra were recorded using a Bruker AMX-400 (400 MHz) instrument. CDCl_3 was used as solvent with tetramethyl silane (TMS) as internal standard and the chemical shifts are given in δ -values. Column Chromatography (CC) was conducted on silica gel (Merck, mesh 80-230). Preparative Thin Layer Chromatography (PTLC, 20X20cm) and Thin Layer Chromatography (TLC, 20X5 cm) were carried out using Merck silica gel 60 PF₂₅₄ on glass plates at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized under UV light (254 and 366 nm) by spraying the developed plates with vanillin-sulfuric acid followed by heating for 5-10 minutes at 110°C. All solvents used in this study were of reagent grade.

Plant materials collection

The stem bark of the plant *Crataeva nurvala* were collected from Natore district of Bangladesh in the month of December, 2007 and identified by Mahbubur Rahman, Assistant Professor Department of Botany, University of Rajshahi, Rajshahi, Bangladesh and its voucher specimen has been deposited in the Herbarium of the same department (Voucher No. 24.11.12, 1965).

Plant material extraction and separation

The stem barks of the plant were cut, air dried for several days and then oven dried for 24 hours below the temperature 60°C. The bark was then pulverized into coarse powder using a grinding machine. The powdered dried stem bark (1.26 kg) was subjected to cold rectified spirit extraction (4.5l) in flat bottom glass container through occasional shaking and stirring for 10 days. The whole extract was filtered through cotton and the solvent was evaporated to dryness in vacuum with rotary evaporator at 40°C to 50°C to afford a semisolid mass (70 gm). This was then diluted with water and fractionated with petroleum ether to remove the fatty matter. The aqueous layer was then extracted with chloroform (thrice). Then the organic layer was dried and the solvent was evaporated off under reduced pressure to afford the chloroform extract as blackish residue (10 gm).

Isolation and Identification

The chloroform extract (10 gm) was further fractionated by column chromatography over silica gel (Merck, mesh 80-230). Elution from the column first extracted with n-hexane, then increasing polarities of EtOAc in n-hexane and finally with EtOAc yielded 37 fractions. The proportion of solvent systems used to obtain fraction 5, 6, 7 and 10 were n-hexane- EtOAc (95:5), (94:6), (93:7) and (90:10) respectively. Fraction 5 gave stigmasterol (4, 23 mg) and fraction 6 gave betulinic acid (1, 20mg) upon multiple

PTLC using n-hexane- EtOAc (95:5) and (94:6) respectively. Repeated crystallization of fraction 7 and fraction 10 using n-hexane- EtOAc afforded lupeol (2, 28 mg) and β -sitosterol (3, 99mg) respectively.

RESULTS

Repeated chromatographic separation and purification of the chloroform soluble materials of the cold rectified spirit extract of the stem bark of *Crataeva nurvala* provided a total of four compounds (compound 1, 2, 3 and 4). The structures of which were determined by extensive ^1H NMR spectral analysis as well as by comparison of their spectral data with previously reported values.

Betulinic acid (1): White mass; ^1H NMR spectrum (400 MHz, CDCl_3): δ 4.65 (1H, d, J=0.4 Hz), 4.55 (1H, d, J=0.4 Hz), 3.16 (dd, J=9.5, 6.0 Hz), 2.95 (ddd, J=9.5, 6.0 Hz, 0.5 Hz), 1.67 (brd, J=0.5 Hz), 1.65 (3H, s, Me -30), 0.98 (3H, s, Me -26), 0.96 (3H, s, Me -23), 0.90 (3H, s, Me -27), 0.75 (3H, s, Me -25), 0.65 (3H, s, Me -24).

Lupeol (2): White crystal; ^1H NMR spectrum (400 MHz, CDCl_3): δ 4.67 (1H, dq, J=0.4, 0.5 Hz, Hb-29), 4.56 (1H, d, J=0.4 Hz, Ha-29), 3.18 (1H, dd, J=9.6, 6.2 Hz, Ha-3), 1.67 (3H, brd, J=0.5 Hz, Me-30), 1.02, 0.94, 0.92, 0.81, 0.78, 0.75 (Me-27, Me-26, Me-25, Me-24, Me-23, Me-28).

β -sitosterol (3): Colorless needles; ^1H NMR spectrum (400 MHz, CDCl_3): δ 5.39 (1H, m, H-6), 3.51 (1H, m, H-3), 1.05 (3H, s, Me-19), 0.96 (3H, d, J=6.5 Hz, Me-21), 0.89 (3H, t, J=7.4 Hz, Me-29), 0.87 (3H, d, J=6.7 Hz, Me -26), 0.85 (3H, d, J=6.7 Hz, Me -27), 0.72 (3H, s, Me-18).

Stigmasterol (4): Colorless amorphous powder; ^1H NMR spectrum (400 MHz, CDCl_3): δ 5.35 (1H, m, H-6), 5.13 (1H, dd, j=14.4, 8.4 Hz, H-22), 5.03 (1H, dd, j=14.4, 8.4 Hz, H-23), 3.51 (1H, m, H-3), 1.0 (3H, s, CH_3 -10), 0.91 (3H, d, J=6.4 Hz, CH_3 -20), 0.85 (3H, d, J=7.4 Hz, CH_3 -27), 0.81 (3H, d, J=7.4 Hz, CH_3 -26), 0.67 (3H, s, CH_3 -13).

DISCUSSION

The ^1H NMR spectra of compound (1) (Fig 1) revealed the presence of a lupene type carbon skeleton. Signals at δ 4.55 and δ 4.65 appeared as one proton doublet with coupling constant 0.4 Hz indicates an exomethylene group. The double doublet at δ 3.16 with coupling constants 9.5 Hz and 6.0 Hz centered at and a doublet of double doublet at δ 2.95 with coupling constant 9.0 Hz, 6.0 Hz and 0.5 Hz centered at could be assigned for a secondary carbinol. The spectrum also showed broad doublet at δ 1.67 with coupling constant 0.5 Hz was indicative of a vinyl methyl group. The spectrum revealed resonance signals at δ 0.65, 0.75, 0.90, 0.96 and 0.98 for five tertiary methyl groups. These data indicated a pentacyclic triterpenoid of betulinic acid and comparison with

published data confirmed the identity of compound 1 as betulinic acid (Nazma et al., 2009).

The ^1H NMR spectrum of the compound (2) (Fig 1) revealed signals at δ 4.56 appeared as one proton doublet with coupling constant 0.4 Hz and signals at δ 4.67 as one proton doublet with coupling constant 0.4 Hz and signals at δ 4.67 as one proton doublet with coupling constant 0.4 Hz and 0.5 Hz centered at for an exomethylene group. The signals at δ 3.18 appeared as double doublets with coupling constant 9.6 Hz and 6.2 Hz centered at could be assigned for a secondary carbinol group. The spectrum also showed a broad doublet at δ 1.66 with coupling constant 0.5 Hz was attributed to a methine group. The resonance signals at δ 0.75, 0.77, 0.80, 0.92, 0.94 and 1.02 exhibited six tertiary methyl singlets. These data were in close agreement with those reported for a typical pentacyclic triterpenoid lupeol (Haque et al., 2007) and further confirmed the identity of compound 2 as lupeol.

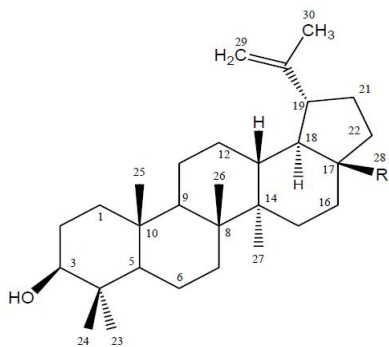


Fig 1: Chemical structure of Betulinic acid (R=COOH) and Lupeol (R=H).

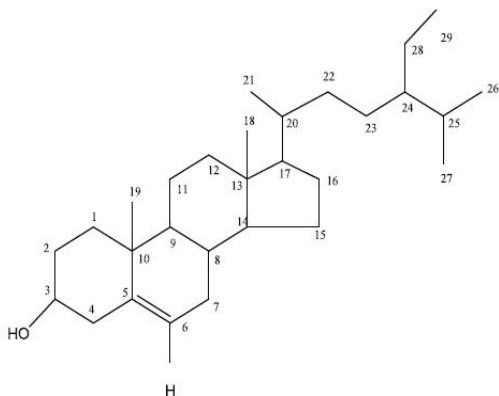


Fig 2: β -sitosterol.

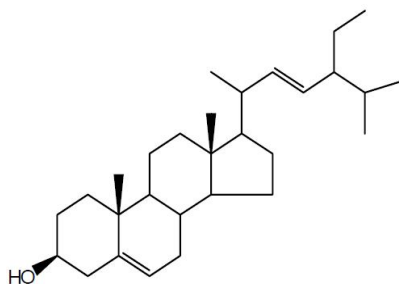


Fig 3: Stigmasterol.

The ^1H NMR spectrum (400 MHz, CDCl_3) of the compound (3) (Fig 2) has revealed a one proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical H-6 of the steroidal skeleton was appeared as a multiplet at δ 5.39 for one proton. The resonance signals at δ 0.72 and δ 1.05 (3H each) was due to two tertiary methyl groups at C-13 and C-10 respectively. Two doublets centered at δ 0.87 with coupling constant 6.7 Hz and δ 0.85 with coupling constant 6.7 Hz were assigned for two methyl groups at C-25. The ^1H NMR spectrum showed a doublet at δ 0.96 with coupling constant 6.5 Hz was for a methyl group at C-20. The spectrum also showed three proton triplet at δ 0.89 could be assigned to the primary methyl group at C-28. On this basis compound (3) was characterized as β -sitosterol, the identity of which was confirmed by comparison of the spectral data with previously reported values (Muhit et al., 2010).

The ^1H NMR spectra of compound (4) (Fig 3) revealed the typical signal for the olefinic H-6 of the steroidal skeleton which was evident from a multiplet at 5.35 integrating for one proton. The olefinic protons (H-22 and H-23) appeared as characteristics downfield signals at δ 5.13 and δ 5.03 respectively. Each of the signal was observed as double doublets ($J=14.4$, 8.4 Hz). The spectrum showed a one proton multiplet at δ 3.51. The position and multiplicity of which was indicative of H-3 of the steroidal nucleus. Signals at δ 0.67 and δ 1.00 (three protons each) could be assigned to two tertiary methyl groups at C-13 and C-10 respectively. Two doublets at δ 0.81 ($J=7.4$ Hz) and 0.85 ($J=7.4$ Hz) which was due to the two methyl groups at C-25. The doublet at δ 0.91 ($J=6.4$ Hz) was due to a methyl group at C-20. These spectral features are in close agreement to those observed for stigmasterol (Muhit et al., 2010). On this basis, the identity of compound (4) was confirmed as stigmasterol.

CONCLUSION

Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds. But it is necessary to isolate the active principles and characterize their constituents for the benefit of human being. It was our attempt to identify the new compounds in this plant that revealed four compounds and all of them are previously established.

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