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Research Article

Isolation and characterization of natural phytoconstituents from stem bark of *Crataeva nurvala*

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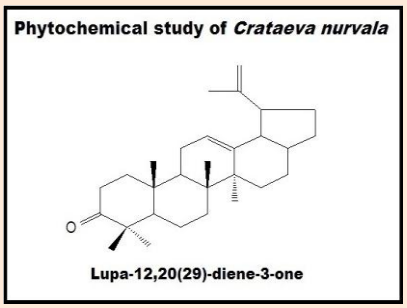
Abstract

Petroleum ether extract of stem bark of *Crataeva nurvala* afforded these natural compounds: Lupeol acetate **(1)**, Lupa-12,20(29)-diene-3-one **(2)**, Lupeol **(3)**, Stigmasterol **(4)** and *β*-sitosterol **(5)**. Characterization of these natural compounds was done on the basis of spectral studies.

Keywords: *Crataeva nurvala*, *Crataeva* genus, Biological activity, Natural compounds, Phytochemicals



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Introduction

Crataeva nurvala (Genus: Crataeva, Family: Capparidaceae) is commonly known as barna and varuna in Hindi and Bengali [1]. It is distributed throughout India and tropical regions of the world cultivated [2, 3]. It is found along streams and also in dry, deep boulder formations in sub Himalayan tract [4]. It is a deciduous and much branched tree with trifoliate, glabrous and ovate leaflets [5]. Phytochemical studies showed that stem bark of the plant contains saponins, flavonoids, sterols, glucosilinates, cerylalcohol, friedelin, cadabicinediacetate, lupeol, betulinic acid and diosgenin [6]. Fruits contain glucocapparin, *β*-sitosterol, triacontane, triacontanol, cetyl and ceryl alcohol [7]. Leaves are reported to contain *L*-stachydrine, dodecanoicanhydride, methyl pentacosanoate, kaemferol-*0*-*α*-D-glucoside and quercitin-3-*0*-*α*-*D*-glucoside [8]. Root bark contains rutin, quercitin, lupeol, varunol and *β*-sitosterol [9]. It is useful as laxative, demulcent, stomachic and urinary troubles [10]. It is also useful as anti-inflammatory drug and act as a good contraceptive for women. This plant is known to possess immense pharmacological activity nephrotoxiciy [11], arthritis [12], lipid peroxidation in adjuvant induced arthritis [13], urolithiasis [14], urinary disorders [15] and antilithic properties. Leaves are externally rubefacient and used in rheumatism, febrifuge and tonic [16]. The major component isolated from this plant is lupeol, which is used to treat hypercrystalluria, hyperoxaluria and hypercalciuria [17, 18]. This compound also decreases elevated concentration of oxalate, phosphorus and magnesium in renal tissue [19].

Experimental

Material and Methods

General Experimental Procedure

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative TLC was conducted on aluminium sheet Kieselgel 60 F254 (E. Merck). Silica gel (E. Merck, 60-120 mesh, 550 gm) used for column (1.5m × 4.0cm) chromatography. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 MHz and 75 MHz on a

Brucker NMR instrument, respectively, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon /Xenon as FAB gas.

Plant material

The plant material (Stem bark) was collected from the locality of Jaipur, Rajasthan and the authenticity of the plant was confirmed by Incharge of Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Extraction and Isolation of the Constituents

The shade dried plant material (5 kg) was finely powdered and extracted with petroleum ether in a 5 liter round bottom flask for (12×3) hrs on water bath. The extract was filtered and the solvent was removed under reduced pressure. To avoid unwanted fat, the extract was treated with acetonitrile and aliquot of acetonitrile phase was transferred into a centrifuge tube and stored in a freezer for two days where the major part of fat (42 g) was precipitated. The precipitate was separated and dried. Acetonitrile phase was also combined filtered and evaporated to dryness where a semi-solid, sticky and yellowish mass (30 g) was obtained. The column was eluted with different solvents in order of increasing polarity where following compounds were isolated, purified and characterized.

Characterization of Lupeol acetate (1)

When column was eluted with petroleum ether compound 1 obtained. After crystallization with methanol, colorless solid was obtained and its melting point was observed 222°C. IR (KBr, cm^{-1}) 1733 (>C=O stretching), 1652 (C=C stretching), 1385, 1370 (gem dimethyl group) and 1050 (C-O stretching); ^{1}H NMR (δ ppm, $CDCl_3$) 4.44 (dd, 1H, C–3), 1.64 (*s*, 3H, C–30), 2.36 (*m*, 2H, C–21), 1.04 (*s*, 3H, C–23), 0.78 (*s*, 3H, C–24), 0.87 (*s*, 3H, C–25), 0.93 (*s*, 3H, C–26), 0.84 (*s*, 3H, C–27), 0.96 (*s*, 3H, C–28), 4.56 (br, *s*, 1H, C-29), 4.68 (br, *s*, 1H, C-29), 2.04 (*s*, 3H, -$OCOCH_3$), 1.25-1.68 (remaining 23 protons); ^{13}C NMR (δ ppm, $CDCl_3$) 38.33 (C–1), 27.38 (C–2), 80.94 (C–3), 37.76 (C–4), 55.33 (C–5), 18.16 (C–6), 34.15 (C–7), 39.96 (C–8), 50.29 (C–9), 37.03 (C–10), 21.32 (C–11), 25.03 (C–12), 37.98 (C–13), 42.96 (C–14), 27.91 (C–15), 35.52 (C–16), 42.77 (C–17), 47.97 (C–18), 48.23 (C–19), 150.96 (C–20), 29.78 (C–21), 40.79 (C–22), 27.38 (C–23), 14.46 (C–24), 17.96 (C–25), 16.47 (C–26), 16.15 (C–27), 19.25 (C–28), 109.33 (C–29), 20.89 (C–30), 171.03 (-O<u>C</u>OCH_3 at C–3), 23.67 (-OCO<u>C</u>H_3 at C–3); MS(m/z) 491 (M^+ + Na), 468 (M^+), 453, 423, 410 (base peak), 391, 385, 327, 281, 175, 161, 147, 135, 121, 107. Molecular formula calculated as $C_{32}H_{52}O_2$.

Characterization of Lupa-12,20(29)-diene-3-one (2)

It was isolated when column was eluted with petroleum ether and benzene in the ratio 3:1 and they were separated by PTLC by using n-hexane: benzene (2:3) as mobile phase. Melting point was found to be 194°C. IR (KBr, cm^{-1}) 1745(>C=O str.), 1610 [C=C at C-20(29)], 1630 [C=C at C-12 (13)]; ^{1}H NMR (δ ppm, $CDCl_3$) 0.80 (*s*, 3H, C-24), 0.87 (*s*, 6H, C-25, C-27), 0.1.14 (*s*, 9H, C-23, C-26, C-28), 1.61 (*s*, 3H, C-20), 4.67, 4.68 (*d*, 2H, C-30), 2.21-2.52 (*m*, 2H, C-2), 1.18-1.58 (remaining 18 protons), 5.04 (br, *s*, 1H, C-12), 1.71 (*d*, 2H, C-2); MS(m/z) 422 (M^+). Molecular formula calculated as $C_{30}H_{46}O$.

Characterization of Lupeol (3)

Compound 3 was isolated when column was eluted with petroleum ether with benzene in the ratio 1:1. The solvent was removed under reduced pressure. The solid product so obtained was crystallized from methanol as slightly yellowish-white powder (m.p. 220°C). IR (KBr, cm^{-1}) 3635(OH stretching), 1650 (>C=C< stretching), 1390 and 1365 (C–H stretching of >CMe_2 group); ^{1}HNMR (δ ppm, $CDCl_3$) 4.68 (*s*, 1H, C-29), 4.55 (*s*, 1H, C-29), 3.19 (*t*, 1H, C-3), 2.35 (*m*, 2H, C-21), 1.68 (*s*, 3H, C-30), 0.97 (*s*, 3H, C-23), 0.92 (*s*, 3H, C-28), 0.84 (*s*, 3H, C-26), 0.81 (*s*, 3H, C-24), 0.77 (*s*, 3H, C-25), 0.75 (*s*, 3H, C-27), 1.24-1.74 (remaining 23 protons); ^{13}CNMR (δ ppm, $CDCl_3$) 38.70 (C-1), 27.45 (C-2), 79.41 (C-3), 39.00 (C-4), 55.23 (C-5), 18.37 (C-6), 34.38 (C-7), 41.02 (C-8), 50.45 (C-9), 37.22 (C-10), 21.03 (C-11), 24.98 (C-12), 38.10 (C-13), 43.08 (C-14), 27.50 (C-15), 35.67 (C-16), 42.86 (C-17), 48.30 (C-18), 48.02 (C-19), 151.33 (C-20), 29.05 (C-21), 40.06 (C-22), 28.00 (C-23), 15.50 (C-24), 16.02 (C-25), 16.15 (C-26), 14.56 (C-27), 18.01 (C-28), 109.74 (C-29), 19.37 (C-30); MS(m/z) 427 (M+H), 426 (M^+). Molecular formula calculated as $C_{30}H_{50}O$.

Characterization of Stigmasterol (4)

Removal of solvent afforded yellow solid, by eluting the column with benzene compound 4 was obtained (m.p. 167°C). It gave positive Liebermann-Burchard sterol and TNM test for unsaturation. IR (KBr, cm^{-1}) 3400-3200 (OH), 1460 (-CH=CH- bending), 1380, 1360, 1260, 1050, 960, 800; ^{1}HNMR (δ ppm, $CDCl_3$) 5.35 (*t*, C-6), 5.05 (*dd*, *J* = 16.0, 10.0 Hz, C-22), 5.15 (*dd*, *J* = 16.0, 10.0 Hz, C-23), 3.50 (*m*, H-3), 0.84 (*t*, *J* = 7.0 Hz, C-29 methyl), 1.00 (*d*, *J* = 7.0 Hz, C-21 methyl), 1.16 (*s*, C-27 methyl), 0.93 (*s*, C-19 methyl), 0.70 (*s*, C-18 methyl); MS(m/z) 412 (M^+), 399 (M-Me^+), 384, 369, 314, 302, 273, 255. Molecular formula Calculated as $C_{29}H_{48}O$.

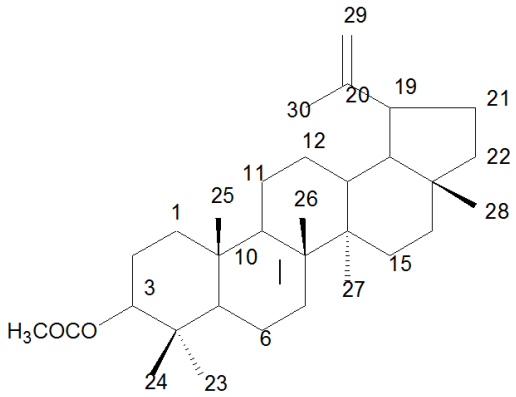
Characterization of β-sitosterol (5)

It was isolated on elution of column with benzene and chloroform in ratio (3:1). On crystallisation with methanol white needle like crystals were obtained. It gave positive Liebermann-Burchard test. It was showed 138°C melting point. IR (KBr, cm^{-1}) 3500-3445 (O–H stretching), 1590 (C=C stretching), 1050 (C-O stretching); ^{1}H NMR (δ ppm, $CDCl_3$) 3.52 (*m*, 1H, C-3), 5.30 (*t*, 1H, C-6), 0.65 (*s*, 3H, C-18), 0.99 (*s*, 3H, C-19), 1.25 (*d*, 3H, C-21), 0.84 (*d*, 3H, C-26), 0.92 (*d*, 3H, C-27), 0.95 (*t*, 3H, C-29), 1.83 (*m*, 1H, C-25), 2.15 (*dd*, 2H, C-7), 1.45-1.85 (*m*, for remaining 26 protons); ^{13}C NMR (δ ppm, $CDCl_3$) 31.30 (C-1), 32.00 (C-2), 72.00 (C-3), 42.20 (C-4), 140.01 (C-5), 122.14 (C-6), 32.02 (C-7), 46.11 (C-8), 49.80 (C-9), 36.12 (C-10), 20.98 (C-11), 28.20 (C-12), 42.34 (C-13), 57.00 (C-14), 24.32 (C-15), 40.12 (C-16), 56.20 (C-17), 12.00 (C-18), 19.50 (C-19), 36.20 (C-20), 19.50 (C-21), 36.15 (C-22), 24.67 (C-23), 39.90 (C-24), 36.00 (C-25), 23.40 (C-26), 23.41 (C-27), 32.20 (C-28), 29.45(C-29); MS(m/z)414 (M^+), 397, 383, 369, 255 etc. Molecular formula calculated as $C_{29}H_{50}O$.

Result and Discussion

Compound 1

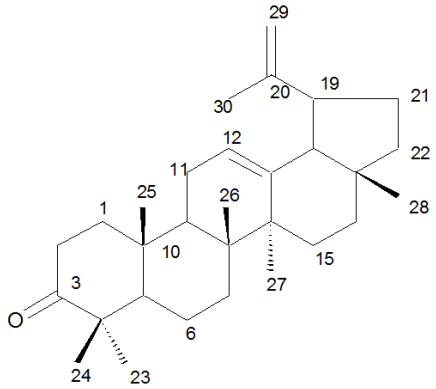
The unsaturated triterpenoid nature of the compound was confirmed by positive tests with Liebermann-Burchard, Noller's reagents and by treatment with TNM. The FAB mass spectrum of the compound showed significant signals at m/z 491 (M^+ + Na), 468 (M^+), 453, 423, 410, 391, 385, 327, 281, 175, 161, 147, 135, 121, 107 etc. On the basis of molecular ion peak the molecular formula for compound 1 was confirmed as $C_{32}H_{52}O_2$. The IR spectrum (KBr, cm^{-1}) of the compound 1 showed sharp absorption at 1733, which confirmed the presence of an acetoxyl group in the molecule. The presence of >C=C< was confirmed by the characteristic absorption at 1652. Absorption at 1050 confirmed the presence of –C–O–C linkage. The other prominent absorptions at 1385 and 1370, were characterized for the presence of gem dimethyl group (>CMe_2) in the title compound. The proton NMR spectrum (δ ppm, $CDCl_3$) showed sharp singlets at 0.78, 0.84, 0.87, 0.93, 0.96 and 1.04 indicated the presence of six methyl groups at C–24, C–27, C–25, C–26, C–28 and C–23 positions respectively. The three protons of methyl group at C–30 position were confirmed by the presence of a sharp singlet at 1.64. The presence of three protons of acetyl group at 2.04 as a sharp singlet confirmed its position at C–3. The proton present at carbon atom C–3 was observed at 4.44 as a double doublet. The presence of two protons of pentacyclic ring at C–21 position was confirmed by a multiplet at 2.36. A pair of broad singlets at 4.56 and 4.68 was assigned to the vinylic protons attached at C–29. The presence of remaining twenty-three protons was calculated in the region from 1.24 to 1.70. In the ^{13}C NMR spectrum (δ ppm, $CDCl_3$), absorptions observed at 27.38 (C–23), 14.46 (C–24), 17.96 (C–25), 16.47 (C–26), 16.15 (C–27) and 19.25 (C–28) confirmed the presence of six methyl groups. The signals observed at 109.33 and 150.96 were assigned for carbon-carbon double bond at C–29 and C–20 carbon atoms respectively. The absorption for methyl group at C–30, which is attached to olifinic carbon atom, appeared at 20.89. The presence of absorption at 80.94 showed the presence of an acetoxy group attached at C–3 position. The absorptions appearing at 171.03 and 23.67 clearly indicated the presence of acetoxyl (–$OCOCH_3$) group. The values of other carbon atoms in compound 1 were established as 38.33 (C–1), 27.38 (C–2), 37.76 (C–4), 55.33 (C–5), 18.16 (C–6), 34.15 (C–7), 39.96 (C–8), 50.29 (C–9), 37.03 (C–10), 21.32 (C–11), 25.03 (C–12), 37.98 (C–13), 42.96 (C–14), 27.91 (C–15), 35.52 (C–16), 42.77 (C–17), 47.97 (C–18), 48.23 (C–19), 29.78 (C–21) and 40.79 (C–22). On the basis of above spectral analysis compound 1 was identified as lupeol acetate [20]. The identity of this compound was further confirmed by comparing the observed values with reported data.



Compound 1: Lupeol acetate

Compound 2

In the mass spectrum of compound 2, prominent molecular ion peak was appeared at m/z 422 [M^+]. The molecular formula of compound was assigned as $C_{30}H_{46}O$ by calculating protons and carbon atoms in the ^1H NMR and ^{13}C NMR spectrum respectively. The IR spectrum (KBr, cm^{-1}) showed characteristic absorption at 1745 suggested the presence of carbonyl group. The presence of >C=C< at C-20(29) and C-12(13) were confirmed by characteristic absorptions at 1610 and 1630 respectively. In the ^1H NMR spectrum (δ ppm, $CDCl_3$) three signals were observed at 0.80 (*s*, 3H), 0.87 (*s*, 6H) and 1.14 (*s*, 9H), were characterized for six tertiary methyl groups. Presence of a singlet at 1.61 confirmed the attachment of methyl group to the olefinic carbon (C-20). A pair of broad singlet at 4.67 and 4.68 for one proton each was attributed for the vinylic protons. A multiplet between 2.21-2.52 was assigned for two protons at C-2 position. A singlet observed at 5.04 for one olefinic proton present at C-12 position. A doublet was observed at 1.71 for two protons at position C-7. A complicated pattern was observed at 1.18-1.58 for eighteen protons. On the basis of above data and observation compound 2 was characterized as lupa-12,20(29)-diene-3-one (m.p. 194°C). The spectral data were compared with reported literature values [21].

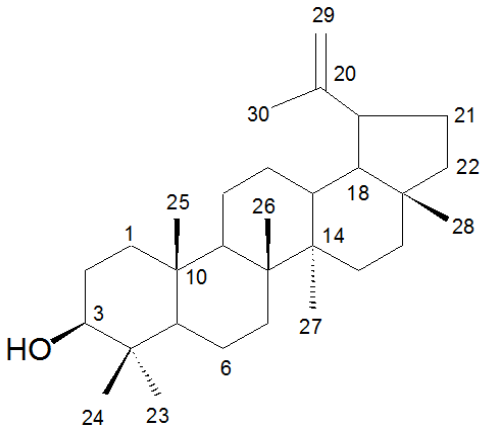


Compound 2: Lupa-12,20(29)-diene-3-one

Compound 3

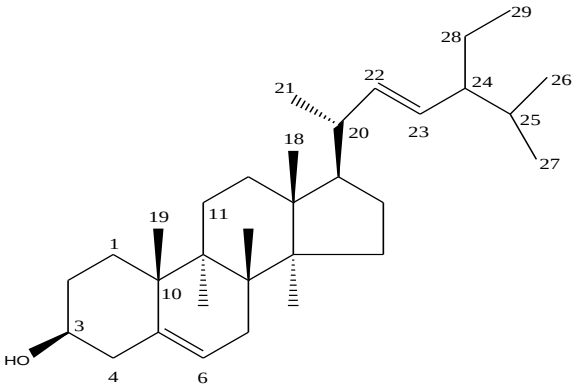
The mass spectrum of compound 3 showed molecular ion peak at m/z 426 (M^+). The molecular formula for compound 3 was assigned as $C_{30}H_{50}O$. It gave positive tests with Liebermann-Burchard and Noller's reagents and thus confirmed its triterpenoid nature. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3635 suggested the presence of hydroxyl group. The presence of >C=C< was confirmed by the characteristic absorption at 1650. The sharp absorptions observed at 1390 and 1365 are characteristic for bending vibrations of gem dimethyl group (>CMe_2). In the proton NMR spectrum (δ ppm, $CDCl_3$) the presence of six tertiary methyl groups were observed at 0.75 (*s*, 3H, C-27), 0.77 (*s*, 3H, C-25), 0.81 (*s*, 3H, C-24), 0.84 (*s*, 3H, C-26), 0.92 (*s*, 3H, C-28) and 0.97 (*s*, 3H, C-23). The methyl group attached to olefinic carbon was observed as a singlet at 1.68 for three protons. The vinylic protons attached at C-29 were observed as a pair of broad singlets at 4.68 and 4.55 for one proton each. The proton

present at C-3 position was observed at 3.19 as a triplet. A multiplet at 2.35 was assigned for two protons present at C-21 position in pentacyclic ring. The remaining twenty-three protons were observed in the region from 1.24 to 1.74. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorptions for olefinic carbon atoms at 109.74 and 151.33. The attachment of hydroxyl group at C-3 position was confirmed by a signal observed at 79.41. Other absorptions observed at 38.70 (C-1), 27.45 (C-2), 39.00 (C-4), 55.23 (C-5), 18.37 (C-6), 34.38 (C-7), 41.02 (C-8), 50.45 (C-9), 37.22 (C-10), 21.03 (C-11), 24.98 (C-12), 38.10 (C-13), 43.08 (C-14), 27.50 (C-15), 35.67 (C-16), 42.86 (C-17), 48.30 (C-18), 48.02 (C-19), 29.05 (C-21), 40.06 (C-22), 28.00 (C-23), 15.50 (C-24), 16.02 (C-25), 16.15 (C-26), 14.56 (C-27), 18.01 (C-28) and 19.37 (C-30) and their assignment has been done accordingly as shown in parentheses. These spectral data are in good agreement with those reported for lupeol in the literature [22-24]. On the basis of these observations compound 3 was identified as lupeol.



Compound 3: Lupeol

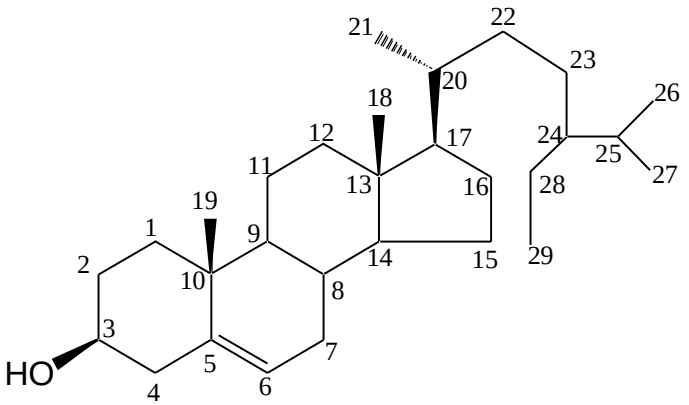
Compound 4 was isolated as shining needles, m.p. 167°C. It gave positive TNM test for unsaturation. IR (cm^{-1}, KBr) spectrum displayed characteristic absorptions at 3400-3200 (OH stretching) and 1460 (-CH=CH-bending). It was analyzed for molecular formula $C_{29}H_{48}O$ [M^+, 412]. The ^1H NMR (δ ppm, $CDCl_3$) spectrum showed a pair of double doublets at 5.05 (*J* = 16.0, 10.0 Hz) and 5.15 (*J* = 16.0, 10.0 Hz) which were explainable to olefinic proton at C-22 and C-23 in the side chain. Large *J* values for these signals indicated the trans orientation of corresponding protons. A broad triplet at δ 5.35 was observed and accounted for C-6 olefinic proton. A multiplet at 3.50 corresponded to C-3 hydroxy methine proton. The singlets at 0.70 (C-18), 0.93 (C-19), 1.16 (C-27), a doublet centered at 1.00 (C-21), and a triplet centered at 0.84 (C-29) for methyl groups in the compound 4 was found similar to those stigmasterol. The chemical shifts for C-18 and C-19 methyl protons were in close agreement with those reported for sterols [25-30]. From the above spectral data, the compound 4 was characterized as stigmasterol and was confirmed by Co-TLC and Co-m.p. with authentic sample [31, 32]



Compound 4: Stigmasterol

Compound 5

In the mass spectrum molecular ion peak was observed at m/z 414 (M^+). Other prominent ions were observed at m/z 397, 383, 369, 255 etc. On the basis of mass spectrum the molecular formula of the compound was established as $C_{29}H_{50}O$. In the IR spectrum (KBr, cm^{-1}) strong absorptions at 3500-3445 (O-H stretching) indicated the presence of hydroxyl group. The absorption at 1590 confirmed the presence of olefinic group (C=C stretching) whereas the absorption at 1050 was assigned for C-O stretching. The proton NMR spectrum (δ ppm, $CDCl_3$) of compound 5 showed a singlet at 0.65 for three protons accounted for tertiary methyl group present at C-18 position. The absorption at 0.84 and 0.92 as a doublets confirmed the presence of methyl protons at C-26 and C-27 positions respectively. A triplet observed at 0.95 was assigned for three protons of two methyl groups present at C-29 position. Methyl protons present at C-19 position showed the absorption at 0.99 as a singlet. The three protons of methyl group present at C-21 position was assigned as a doublet at 1.25. A multiplet was observed at 1.83 for methine proton present at C-25 position. Methylene protons at C-7 appeared as double doublets at 2.15. The olefinic proton present at C-6 was assigned as a triplet at 5.30 with coupling constant *J = 2.8 Hz*. A multiplet observed at 3.52 accounted for one proton and was assigned for a methine proton at C-3 position where the hydroxyl group is attached. The chemical shift and coupling constant *J = 5.60 Hz* of methine proton supported *β*-orientation of hydroxyl (–OH) group at C-3 position. Absorption at 72.00 in ^{13}C NMR spectrum (δ ppm, $CDCl_3$) also confirmed the presence of hydroxyl group at C-3 position. Olefinic carbon atoms were confirmed by the absorptions at 140.01 and 122.14 which were assigned to C-5 and C-6 carbon atoms respectively. Thus confirming the presence of C=C between carbon atom five and six. Other signals were obtained at 31.30 (C-1), 32.00 (C-2), 42.20 (C-4), 32.02 (C-7), 46.11 (C-8), 49.80 (C-9), 36.12 (C-10), 20.98 (C-11), 28.20 (C-12), 42.34 (C-13), 57.00 (C-14), 24.32 (C-15), 40.12 (C-16), 56.20 (C-17), 36.20 (C-20), 36.15 (C-22), 24.67 (C-23), 39.90 (C-24), 36.00 (C-25), 32.20 (C-28), 12.00 (C-18), 19.50 (C-19), 19.50 (C-21), 23.40 (C-26), 23.41 (C-27) and 29.45 (C-29) and their arrangements was done according to the reported values. The above data were found to be similar with those reported for *β*-sitosterol [23]. On the basis of above spectral studies compound 5 was characterized as *β*-sitosterol.



Compound 5: *β*-sitosterol

Acknowledgement

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