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Isolation of Natural Chemical Constituents from aerial part of *Solanum surattens*

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ABSTRACT

Phytochemical examination of aerial part of Solanum surattens afforded 3-oxo-oleane-9(11)-ene (I), lupeol acetate (II), lupa-12(13),20(29)-diene-3-one (III), stigmasterol (IV), β-sitosterol (V) and tomatidenol (VI). Characterization of these compounds was done on the basis of spectral studies.

Key words: *Solanum sursttens*, Solanum species, Biological activity, Phytochemicals,

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INTRODUCTION

Solanaceae is one of the largest and best-known genera of flowering plants. Solanum (potato genus), comprises 1,450 species and is a large, variable genus of annual and perennial plants, vines, sub-shrubs, shrubs and small trees. They often have attractive fruits and flowers. Solanum species have attracted the attention of researchers as they are rich source of precursors of steroidal drugs. Steroidal raw materials have been found useful in cardiovascular therapy, anti-inflammatory agents, menopause regulants and are now known to influence the central nervous system (CNS) [1]. Solanum species have been investigated chemically for their steroidal sapogenin and alkaloid contents in quantitative yield [1]. Plants of Solanum species are employed as tonics, antirheumatics, remedies for colds, fevers, dizziness, anticonvulsants and are eaten as vegetables for their high nutritive values. Solanum species are also reported for their antiviral, anticancer, anticonvulsant and anti-infective activities [1]. Anticonvulsant activity is directly related to the concentration of scopoletin and related coumarin compounds present in most of the Solanum species. The anticonvulsant, sedative, hypotensive and antipyretic properties of scopoletin and scoparone have been reported by many researchers [1]. *Solanum surattens* (Syn.: *Solanum xanthocarpum*) a prickly spreading herb, belongs to family Solanaceae. Its flowers are purple and berries are yellow when ripe. It is distributed throughout India in waste places and road side between 1000 to 2000 meters. Seeds of Solanum species showed antibacterial activity [2]. Solasodine was identified as an antispermatogenic/anti-androgenic principle [3].

MATERIALS AND METHODS

General experimental procedures

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative and quantitative TLC was conducted on aluminium sheet Kieselgel 60 F_{254} (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 (aerial part) was collected from the locality of village Bassi, District Jaipur, Rajasthan and the authenticity of the plant was confirmed by Mr. R. Singh, Incharge of Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Extraction and Isolation of the Constituents

The shade dried plant material (1.5 kg) was finely powdered and extracted with methanol in a 5 litre round bottom flask for 72 hrs on water bath. The extract was filtered hot and solvent was removed by distillation under reduced pressure where a semi-solid dark green mass (27g) was obtained. The solvent free extract was chromatographed over silica gel column. The column was eluted with different solvents in order of increasing polarity where following compounds **(I to VI)** were isolated, purified and characterized.

3-oxo-oleane-9(11)-ene (I)

It was isolated by eluting the column with petroleum ether. After removal of solvent compound I, was crystallized from ethanol where a white crystalline solid was obtained. It gave positive tests with Liebermann-Burchard and Noller's reagents. It also gave yellow colour with TNM, which showed unsaturation in the compound. It showed melting point 229^{o}C. IR (KBr, cm^{-1}): 1700(>C=O str.), 1610(>C=C< str,), 1385(C-H bend. of $CHMe_2$), 1360(C-H bend. of $CHMe_2$); ^{1}H NMR (δ ppm, $CDCl_3$): 0.84 (*s*, 3H, C-28), 0.93 (*s*, 6H, C-25, C-26), 0.96 (*s*, 3H, C-27), 1.05 (*s*, 6H, C-29, C-30), 1.16 (*s*, 3H, C-24), 1.27 (*s*, 3H, C-23), 1.35-2.16 (21H), 2.40-2.55 (*m*, 2H) and 5.65 (*dd*,1H); ^{13}C NMR (δ ppm, $CDCl_3$): 38.42 (C-1), 33.65 (C-2), 180.03 (C-3), 48.92 (C-4), 55.86 (C-5), 38.96 (C-6), 33.16 (C-7), 40.75 (C-8), 157.71 (C-9), 36.95 (C-10), 117.29 (C-11), 37.66 (C-12), 47.62 (C-13), 39.49 (C-14), 26.23 (C-15), 29.75 (C-16), 33.32 (C-17), 48.82 (C-18), 42.00 (C-19), 33.43 (C-20), 28.88 (C-21), 35.87 (C-22), 28.10 (C-23), 21.57 (C-24), 16.40 (C-25), 16.00 (C-26), 14.91 (C-27), 25.63 (C-28), 29.96 (C-29) and 29.10 (C-30); MS (m/z): 424(M^+), 409, 394, 379, 218, 203. Molecular formula calculated as $C_{30}H_{48}O$

Lupeol acetate (II)

When column was eluted with petroleum ether and benzene in the ratio of 3:1, fraction no. 2 was obtained and compound II and III was separated through TLC (n-hexane : benzene :: 2 : 3). After removal of solvent colourless solid was obtained, which was crystallized with methanol 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, -$OCOCH_3$), 1.24-1.70 (remaining 23 protons); ^{13}C NMR (δ ppm, $CDCl_3$): 38.33 (C–1), 27.30 (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 $C_{32}H_{52}O_2$

Lupa-12(13),20(29)-diene-3-one (III)

It was isolated with compound II when elution of column by 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), 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 $C_{30}H_{46}O$

Stigmasterol (IV)

Removal of solvent afforded yellow solid, by eluting the column with petroleum ether and benzene (1:1) compound IV 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-3α), 0.84 (*t*, *J = 7Hz*, C-29 methyl), 1.00 (*d*, *J = 7Hz*, 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^+] ($C_{29}H_{48}O$), 399 [M-Me^+] 384, 369, 314, 302, 273, 255. Molecular formula calculated as $C_{29}H_{48}O$

β-sitosterol (V)

It was isolated on elution of column with benzene. On crystallisation with methanol white needle like crystals were obtained. It gave positive Liebermann-Burchard test. It's showed m.p. 138°C. IR (KBr, cm^{-1}): 3500-3445 (–OH 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),

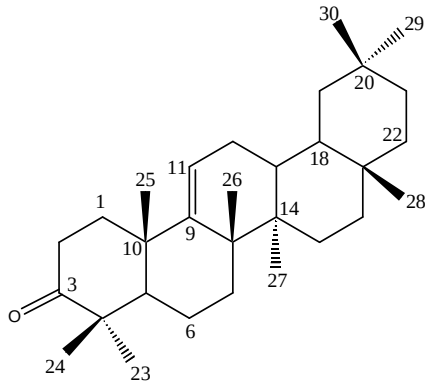
12.00 (C-18), 19.50 (C-19), 36.20 (C-20), 19.50 (C-21), 36.15 (C-22), 24.67 (C-23), 39.90 (C-24), 36.00 (C-25), 23.40 (C-26), 23.41 (C-27), 32.20 (C-28), 29.45 (C-29); MS (m/z): 414 (M^+), 397, 383, 369, 255 etc. Molecular formula calculated as $C_{29}H_{50}O$

Tomatidenol (VI)

Compound VI was obtained when column was eluted with chloroform and methanol in the ratio of 4:1. After removal of solvent colorless solid was obtained which was crystallized from ethyl acetate. Melting point of this compound was found to be 232°C. IR (KBr, cm^{-1}): 3350 (O–H or C-N stretching), 1630 (C=C stretching), 1065 (C-O or C-N stretching); ^{1}H NMR (δ ppm, $CDCl_3$): 0.82 (*s*, 3H, C-18), 1.01 (*s*, 3H, C-19), 0.83 (*d*, 3H, *J = 6.2 Hz*, C-27), 0.95 (*d*, 3H, *J = 6.7 Hz*, C-21), 1.15 to 2.65 (many overlapped peaks), 2.7 (*m*, 2H, C-26), 3.47 (*m*, 1H, C-3), 4.12 (*m*, 1H, C-16), 5.32 (*d*, 1H, *J = 5.8 Hz*, C-65); ^{13}C NMR (δ ppm, $CDCl_3$): 36.90 (C-1), 31.60 (C-2), 71.70 (C-3), 42.30 (C-4), 141.00 (C-5), 121.00 (C-6), 32.10 (C-7), 31.40 (C-8), 50.10 (C-9), 37.3 (C-10), 20.90 (C-11), 40.00 (C-12), 40.60 (C-13), 56.00 (C-14), 32.80 (C-15), 78.50 (C-16), 61.90 (C-17), 16.80 (C-18), 19.40 (C-19), 43.00 (C-20), 15.90 (C-21), 99.10 (C-22), 26.70 (C-23), 28.60 (C-24), 31.10 (C-25), 50.20 (C-26), and 19.40 (C-27); MS (m/z): 413 (M^+). Molecular formula calculated as $C_{27}H_{43}NO_2$

RESULTS AND DISCUSSION

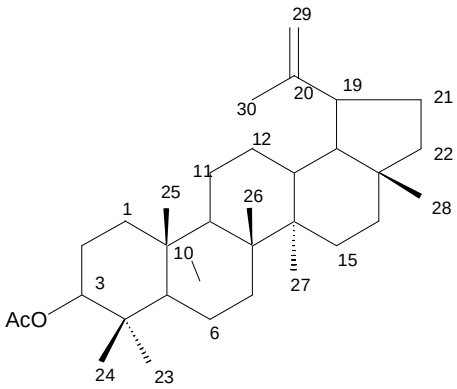
Compound I, in the mass spectrum the molecular ion peak was obtained at m/z 424 indicated the molecular formula of compound I as $C_{30}H_{48}O$. The triterpenoid nature of this compound was confirmed by Liebermann-Burchard [4] and Noller's [5] reagents. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 1700 suggesting the presence of carbonyl group. The presence of >C=C< was confirmed by characteristic absorption at 1610. Strong absorptions observed at 1385 and 1360 were due to the bending vibration of gem dimethyl group (-$CHMe_2$). In the ^{1}H NMR spectrum (δ ppm, $CDCl_3$) presence of six singlets for methyl groups at 0.84 (*s*, 3H), 0.93 (*s*, 6H), 0.96 (*s*, 3H), 1.05 (*s*, 6H), 1.16 (*s*, 3H) and 1.27 (*s*, 3H) were observed. Multiplet observed at 2.40 to 2.55 for two deshielded protons were assigned for H-2eq and H-2ax respectively which is characteristics for 3-keto triterpenoid compounds. A double doublet at 5.65 was assigned for one olefenic proton at C-11 position. From 1.35 to 2.16 complicated patterns was observed and accounted for remaining 21 protons. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) of compound showed characteristic absorption at 117.29, 157.71 and 180.03 suggesting the presence of one carbon-carbon double bond and a ketonic group. These types of absorptions are characteristic for an oxo-oleane triterpenoid. The downfield shift of carbon atoms at C-2 and C-4 were found different as compared to β-amyrin [6], thus indicating the possibility of ketonic group at C-3 position. The absorption at 117.29 and 157.71 are typically observed for C-11, C-9 oleane triterpenoids suggested the presence of double bond between C-9 and C-11 carbon atoms. The absorption for C-2 and C-4 were observed at 33.65 and 48.92 respectively. Other signals were observed at 38.42 (C-1), 33.65 (C-2), 48.92 (C-4), 55.86 (C-5), 38.96 (C-6), 33.16 (C-7), 40.75 (C-8), 36.95 (C-10), 37.66 (C-12), 47.62 (C-13), 39.49 (C-14), 26.23 (C-15), 29.75 (C-16), 33.32 (C-17), 48.82 (C-18), 42.00 (C-19), 33.43 (C-20), 28.88 (C-21), 35.87 (C-22), 28.10 (C-23), 21.57 (C-24), 16.40 (C-25), 16.00 (C-26), 14.91 (C-27), 25.63 (C-28), 29.96 (C-29) and 29.10 (C-30) and their arrangements were done accordingly [7, 8]. The above spectral data were found in good agreement with those reported [7, 8] for 3-oxo-oleane-9(11)-ene. On the basis of above observation compound I was identified as 3-oxo-oleane-9(11)-ene.



Compound I

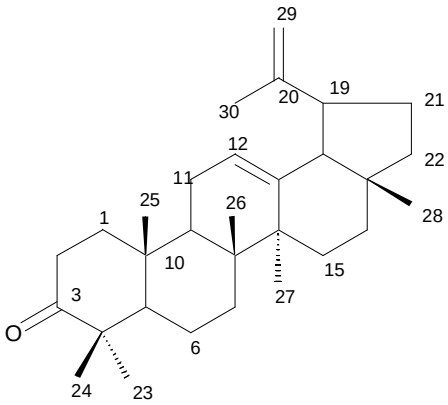
Compound II, the unsaturated triterpenoid nature of the compound was confirmed by positive tests with Liebermann-Burchard, Noller's reagents and by treatment with TNM. The FAB mass spectrum of the compound showed significant signals at m/z 491 (M^+ + Na), 468 (M^+), 453, 423, 410, 391, 385, 327, 281, 175, 161, 147, 135,

121, 107 etc. On the basis of molecular ion peak the molecular formula for compound II was confirmed as $C_{32}H_{52}O_2$. The IR spectrum (KBr, cm^{-1}) of this compound 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 II were established as 38.33 (C–1), 27.30 (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 II was identified as lupeol acetate [9]. The identity of this compound was further confirmed by comparing the observed values with reported data [9].



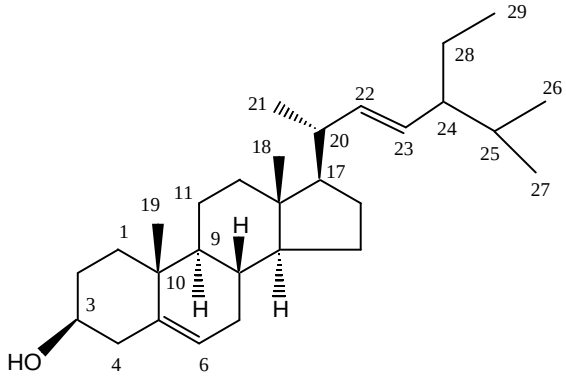
Compound II

Compound III, in the mass spectrum of compound III, 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-2. A complicated pattern was observed at 1.18-1.58 for eighteen protons. On the basis of above data and observation compound III was characterized as lupa-12(13),20(29)-diene-3-one (m.p. 194°C). The spectral data were compared with reported literature values [10].



Compound III

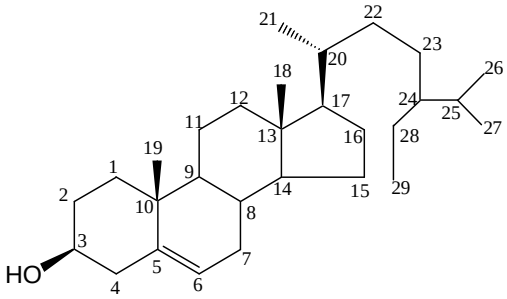
Compound IV, compound IV was isolated as powder material, m.p. 167°C. It gave positive TNM test for unsaturation. IR (KBr, cm^{-1}) spectrum displayed characteristic absorptions at 3400-3200 (-OH stretching) and 1460 (-CH=CH- bending). It was analyzed for molecular formula $C_{29}H_{48}O$ [M^+, 412]. 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 IV was found similar to stigmasterol. From the above spectral data, the compound IV was characterized as stigmasterol and was confirmed by Co-TLC and Co-m.p. with authentic sample [11, 12].



Compound IV

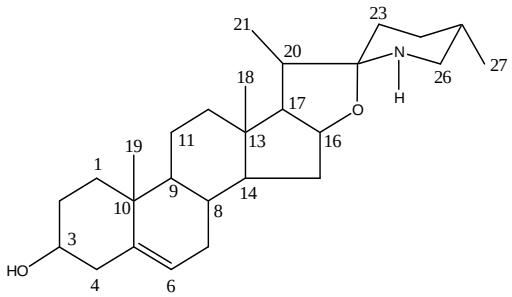
Compound V, In the mass spectrum molecular ion peak was observed at m/z 414 (M^+). Other prominent ions were observed at m/z 397, 383, 369, 255 etc. On the basis of mass spectrum the molecular formula of the compound was established as $C_{29}H_{50}O$. In the IR spectrum (KBr, cm^{-1}) strong absorptions at 3500-3445 (-OH 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 this compound 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 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 were 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. An 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 [13, 14]. On the basis of above spectral studies compound V was characterized as β-sitosterol.



Compound V

Compound VI, the mass spectrum showed the molecular ion at m/z 413 (M^+). The ^{1}H NMR spectrum indicated that it contains fourty three protons and ^{13}C NMR indicated twenty seven carbons in the skelton. On the basis of these observations the molecular formula of compound IV was calculated as $C_{27}H_{43}NO_2$. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3350 (-OH or -NH stretching). The absorption at 1630 confirmed the presence of olefinic group (C=C stretching) whereas the absorption at 1065 was assigned for C-O stretching or C-N stretching. In the ^{1}H NMR spectrum (δ ppm, $CDCl_3$), the presence of four methyl groups were observed at 0.82 (*s*, 3H, C-18), 1.01 (*s*, 3H, C-19), 0.83 (*d*, 3H, *J = 6.2 Hz*, C-27) and 0.95 (*d*, 3H, *J = 6.7 Hz*, C-21). The proton attached at C-6 was observed as a triplet at 5.32 for one proton. The proton adjacent to hydroxyl group at C-3 position was observed at 3.47 as a multiplet. A multiplet at 4.12 was assigned for one proton present at C-16 position. Two protons adjacent to nitrogen in hexacylic ring at position C-26 were observed as a multiplet. The remaining twentyfour protons were observed in the region from 1.15 to 2.65. An absorption at 71.70 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 141.00 and 121.00 which were assigned to C-5 and C-6 carbon atoms respectively. Other signals were obtained at 36.90 (C-1), 31.60 (C-2), 42.30 (C-4), 32.10 (C-7), 31.40 (C-8), 50.10 (C-9), 37.3 (C-10), 20.90 (C-11), 40.00 (C-12), 40.60 (C-13), 56.00 (C-14), 32.80 (C-15), 78.50 (C-16), 61.90 (C-17), 16.80 (C-18), 19.40 (C-19), 43.00 (C-20), 15.90 (C-21), 99.10 (C-22), 26.70 (C-23), 28.60 (C-24), 31.10 (C-25), 50.20 (C-26), and 19.40 (C-27). On the basis of above observations and discussion compound VI was identified as tomatidenol [15].



Compound VI

CONCLUSION

The objective of the present work was to find the medicinal importance of the plant. By using chromatography techniques, we isolate six phyto-constituents and they are identified and characterized on the basis of different spectroscopic techniques. These compounds have very useful medicinal activities. So present work gives a direction for future investigators to carry out research on phytochemistry, so that they could get some medicinally important drugs.

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