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IDENTIFICATION OF NOVEL NATURAL MOLECULE FROM METHANOLIC STEM BARK EXTRACT OF *PARKINSONIA ACULEATA*

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ABSTRACT: *Parkinsonia aculeata* (family - Caesalpiaceae) is a large spinous shrub found in warmer regions of the world. It is a well known medicinal plant that has significant and promising biological activities such as antioxidant, anti-inflammatory, antidiabetic, antispermatogenic, etc. The phytochemical investigations of the leaves, flowers, and stem have revealed the presence of various glycosides, flavonoids, reducing sugars, alkaloids, sterols, tannins, volatile oils, and saponins. The various biological activities and potent phytoconstituents of the plant indicate its therapeutic potential efficacy. The present study shows the detailed investigation of the methanolic stem bark extract of the title plant. From the methanolic stem bark extract of the title plant 1-cyclohexyltridec-1-ene (new compound A), 6-hydroxypentacosylpentanoate (B), α -amyrin acetate (C), α -amyrin (D) and β -sitosterol (E) were isolated with the help of column chromatographic technique and characterized on the basis of different spectral studies i.e., ¹H NMR, ¹³C NMR, IR, and Mass spectroscopy.

INTRODUCTION: A wide variety of flora has been provided to mankind by nature which is the source of biologically active phytoconstituents having profound therapeutic properties. These phytoconstituents can act as templates for green medicines which are the safer alternative of synthetic drugs. Green medicines have better efficacy and less side effects in comparison to their synthetic counterparts. The title plant has been investigated to explore its phytoconstituents for further future application as potential drugs.

Parkinsonia aculeata is a decorative and shade tree of the Caesalpiaceae family. It is a small genus comprising of only one species i.e. *Parkinsonia aculeata*, which is abundantly available in tropical America and also is recently naturalized in different regions of India. It is commonly known as Vilayati Babool, Jerusalem thorn, Mexican paloverdo, and Jellybean tree. Seeds of *Parkinsonia aculeata* have been analyzed for their protein content as well as for fiber, carbohydrate, fixed oil, fatty acid profile and trypsin inhibiting activity ¹.

A water-soluble galactomannan has been characterized by *Parkinsonia aculeata* seeds ². The endosperm of seeds has been reported to possess a water-soluble galactomannan having galactose and mannose in the ratio ³ 1: 2.66. The major proteins of the seed bulk of *Parkinsonia aculeata* are glutelin and albumin.

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The alcoholic extract of the bark showed central nervous system depressant activity in mice and its aqueous extract was found to possess cholinomimetic activity. The literature survey of the genus *Parkinsonia* also shows the presence of flavone-C-glycosides⁴⁻⁷, furfural contents, carbohydrates, amino acids, and fatty acids. A novel flavone glycoside scutellarein-7-*O*-rutinoside along with two known compounds *i.e.* tricin-7- β -L-arabinoside and lupeol were isolated from the flowers⁸. The chemical constituents from the leaf extract⁹ of the title plant were orientin, iso-orientin, vitexin,^{10, 11} isovitexin, leucenin-II, vicenin-II, apigenin, luteolin, kaempferol, chrysoeriol, diosmotin-6- β -glucoside, a new flavanone, parkintin with epoxy isopentyl moiety and permethylated 5-C-hexosylluteolin. *P. aculeata* was reported to possess antimicrobial,¹² antigenotoxic,¹³ antidiabetic,^{14, 15} antioxidant,¹⁶ antimutagenic,¹⁷ antiinflammatory,¹⁸ hepato-protective¹⁹ and antitumor activities^{20, 21}.

MATERIALS AND METHODS:

General Experimental Procedure: FTIR Nicolet Magna 550 and Shimadzu 8400 S spectrometers were used to record IR spectra with KBr pellets. JEOL AL 300 MHz FT-NMR spectrometer was used for the measurement of ¹H NMR and ¹³C NMR spectra and CDCl₃ as solvent. JEOL SX-102 spectrometer was used to record mass spectra (FAB-MS). For qualitative and quantitative TLC, aluminum sheet Kieselgel 60 F₂₅₄ (E. Merck) was used. Electrothermal melting point apparatus was used for the determination of melting point and are uncorrected.

Plant Material: The plant material (stem bark) of *P. aculeata* was collected from Jaipur, Rajasthan, India. The authentication of the plant material was done by the Herbarium Incharge, Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen of RUBL/201478 was submitted to the herbarium of the University.

Extraction and Isolation of the Chemical Constituents: The stem bark was subjected to shade drying and then a coarse powder was obtained (2.5 kg). The extraction was done with the help of methanol for 48 h on a water bath. The extract was concentrated under reduced pressure where a semisolid syrupy substance was obtained (22.0 g). The extract was treated with acetonitrile to

remove fat. The solvent free extract was subjected to column chromatographic separation over a silica gel column. Elution of the column was done by the solvents and different solvent systems were used according to increasing polarity order. The following compounds were isolated and characterized with the help of various spectroscopic data.

1- Cyclohexyltridec- 1- ene (Compound A):

Compound A was obtained when the column was eluted with petroleum ether and benzene in the ratio (3:1). After the removal of the solvent under reduced pressure a white solid was obtained. After treatment with acetone white amorphous powder was obtained with m.p. 70 °C. Molecular formula C₁₉H₃₆; MS (m/z) 265 [M⁺]; IR (KBr, cm⁻¹) 2907, 2840 (C-H str); ¹H NMR (δ ppm CDCl₃) 5.64 (dd, 1H, C-1), 5.30 (m, 1H, C-2), 1.93 (m, 2H, C-3), 1.27 (br, s, for methylene protons), 0.86 (t, 3H, C-12).

6-Hydroxypentacosylpentanoate (Compound B):

It was obtained when the column was eluted with benzene and chloroform in the ratio (3:1). Crystallization was done with methanol which yielded white compound with m.p. 84°C. Molecular formula C₃₀H₆₀O₃; MS (m/z) 468 (M⁺), 453, 116, 73 etc; IR (KBr, cm⁻¹) 3385 (O-H str), 1738 (>C=O str), 1135 (C-O str), 1465 (C-C str), 1060 (C-O-C str); ¹H NMR (δ ppm CDCl₃) 4.13 (t, 2H, CO-O-CH₂), 1.23 (br, 40 H), 2.26 (t, 2H, CH₂-CO-O), 0.84 (t, 6H, 2-Me).

α -Amyrin acetate (Compound C):

Eluting the column with benzene and chloroform in the ratio 1:1 afforded a light yellow solid. This fraction after removal of solvent was crystallized by acetone as colourless solid. It was identified as α -amyrin acetate by Co-TLC with authentic sample. Molecular formula C₃₂H₅₂O₂; MS (m/z) 468 (M⁺), 453, 409, 218, 203, 189, 135, 105; IR (KBr, cm⁻¹) 1739 (C=O str), 1650 (C=C str), 1050 (C-O str), 1385, 1375 (>C(CH₃)₂); ¹H NMR (δ ppm CDCl₃) 0.80 (s, 3H, C-25), 0.86 (s, 3H, C-26), 0.78 (s, 3H, C-24), 0.95 (d, 3H, C-29), 0.92 (d, 3H, C-30), 0.97 (s, 3H, C-27), 1.03 (s, 3H, C-23), 1.07 (s, 3H, C-28), 4.55 (t, 1H, C-3), 2.04 (s, 3H, C-3), 5.15 (s, 1H, C-12), 1.28-1.93 (remaining 23 protons); ¹³C NMR (δ ppm, CDCl₃) 38.55 (C-1), 23.01 (C-2), 78.95 (C-3), 37.25 (C-4), 55.55 (C-5), 18.04 (C-6),

23.25 (C-7), 40.52 (C-8), 47.95 (C-9), 37.00 (C-10), 17.85 (C-11), 123.55 (C-12), 138.85 (C-13), 42.00 (C-14), 28.24 (C-15), 37.85 (C-16), 33.08 (C-17), 59.45 (C-18), 40.46 (C-19), 39.00 (C-20), 31.51 (C-21), 41.65 (C-22), 28.14 (C-23), 16.85 (C-24), 15.06 (C-25), 17.15 (C-26), 24.05 (C-27), 28.10 (C-28), 22.95 (C-29), 20.85 (C-30), 171.25 (O-CO-CH₃), 23.6 (OCOCH₃).

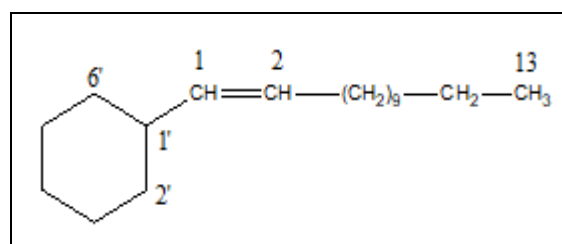
α -Amyrin (Compound D): When the column was eluted with benzene and chloroform in the ratio (1:1), compound D was obtained. Crystallization after removal of the solvent afforded colourless crystals with m.p. 186 °C. Molecular formula C₃₀H₅₀O; MS (m/z) 426 (M⁺), 427 (M⁺H), 409, 341, 327, 313, 271, 218, 203, 135, 119; IR (KBr, cm⁻¹) 3250-3200 (O-H str), 1755 (>C=O str), 1385, 1370 (>C(CH₃)₂), 1060 (C-O str); ¹H NMR (δ ppm CDCl₃) 0.95 (s, 3H, C-23), 0.76 (s, 3H, C-24), 0.82 (s, 3H, C-25), 0.79 (s, 3H, C-26), 1.06 (s, 3H, C-27), 1.04 (s, 3H, C-28), 0.88 (d, 3H, C-29), 0.86 (d, 3H, C-30), 4.43 (t, 1H, C-3), 1.25-2.10 (remaining 24 protons); ¹³C NMR (δ ppm, CDCl₃) 38.43. (C-1), 23.72 (C-2), 79.23 (C-3), 37.54 (C-4), 55.61 (C-5), 18.55 (C-6), 23.67 (C-7), 40.48 (C-8), 48.20 (C-9), 37.32 (C-10), 17.63 (C-11), 124.56 (C-12), 139.87 (C-13), 42.36 (C-14), 28.42 (C-15), 37.12 (C-16), 33.23 (C-17), 59.37 (C-18), 40.05 (C-19), 39.22 (C-20), 31.50 (C-21), 41.34 (C-22), 28.32 (C-23), 16.52 (C-24), 15.61 (C-25), 16.84 (C-26), 23.67 (C-27), 28.57 (C-28), 23.44 (C-29), 21.45 (C-30).

β -sitosterol (Compound E): On eluting the column with chloroform, β -sitosterol was obtained. The crystallization of the compound was done with methanol, which yielded white needle like crystals. The compound E also gave positive Liebermann-Burchard test and m.p. was observed as 137°C. Molecular formula C₂₉H₅₀O; MS (m/z) 414 (M⁺), 397, 383, 369, 255; IR (KBr, cm⁻¹) 3500-3440 (O-H str), 1580 (C=C str), 1050 (C-O str); ¹H NMR (δ ppm CDCl₃) 3.52 (m, 1H, C-3), 5.30 (t, 1H, C-6), 0.65 (s, 3H, C-18), 0.98 (s, 3H, C-19), 1.24 (d, 3H, C-21), 0.83 (d, 3H, C-26), 0.94 (d, 3H, C-27), 0.96 (t, 3H, C-29), 1.83 (m, 1H, C-25), 2.14 (dd, 2H, C-7), 1.45-1.85 (m, for remaining 26 protons); ¹³C NMR (δ ppm, CDCl₃) 31.32 (C-1), 32.00 (C-2), 72.00 (C-3), 42.22 (C-4), 140.02 (C-5), 122.15 (C-6), 32.03 (C-7), 46.14 (C-8), 49.80 (C-9), 36.14 (C-10), 29.95 (C-11), 28.22 (C-12), 42.36 (C-13),

57.00 (C-14), 24.34 (C-15), 40.14 (C-16), 56.25 (C-17), 12.00 (C-18), 19.54 (C-19), 36.23 (C-20), 19.53 (C-21), 36.16 (C-22), 24.65 (C-23), 39.92 (C-24), 36.01 (C-25), 23.42 (C-26), 23.44 (C-27), 32.20 (C-28) and 29.47 (C-29).

RESULTS AND DISCUSSION:

Compound A (New Compound): The molecular formula of the compound was established with the help of mass spectral data as C₁₉H₃₆. The molecular ion peak in the mass spectrum appeared at m/z 265[M⁺+1]. Other important ions observed in the mass spectrum are 250, 236, and 123. It was obtained as a white powder. Compound A gave positive tetra-nitromethane (TNM) test for unsaturation. The IR spectrum (KBr, cm⁻¹) showed characteristic absorptions at 2920 cm⁻¹ and 2848 cm⁻¹ for C-H stretching and 1470 cm⁻¹ for -CH₂-deformation. The presence of >C=C< was confirmed by the characteristic absorption at 1615. The ¹H NMR spectrum (δ ppm, CDCl₃) displayed a double doublet at 5.64 and a multiplet at 5.30 for one proton each which accounts for the olefinic protons at C-1 and C-2 positions respectively. A broad signal at 1.54 was assigned to the protons present at C-2', C-3', C-4' C-5' and C-6' positions of cyclohexyl ring. Whereas the methylene protons adjacent to olefinic bond made their appearance at 1.93 as a multiplet. The signal corresponding to terminal methyl group appeared at 0.86 as a triplet. The rest of the eighteen protons of the methylene groups appeared as a broad singlet at 1.27. On the basis of the above evidences, compound A was identified as 1-cyclohexyltridec-1-ene. Isolation and characterization of compound A are being reported for the first time.

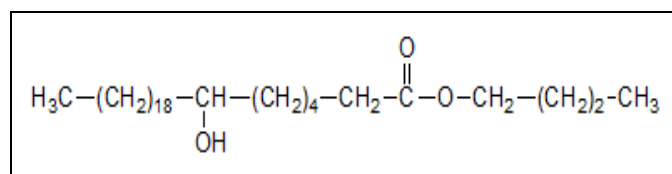


COMPOUND A

Compound B: In the mass spectrum of the compound the molecular ion peak was observed at m/z 468 (M⁺) and its molecular formula was calculated as C₃₀H₆₀O₃ on the basis of mass spectral data. The mass spectrum of the compound gave significant evidence about the structure. In its mass

spectrum, molecular ion peak was observed at 468 (M^+) and the spectrum showed other significant ions at m/z 453, 116 and 73. The asymmetrical nature of side-chain was confirmed by the absence of $[M^+-15]$ ion and the presence of a peak corresponding to (M^++1) ion. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3385 (br), indicated the presence of hydroxy group. The absorption at 1738 confirmed the presence of $>C=O$ group. The presence of the C-O group was confirmed by the characteristic absorption at 1135.

The absorption signal corresponding C-O-C stretching appeared at 1060 and C-C stretching was observed at 1465. In the 1H NMR spectrum (δ ppm, $CDCl_3$) a triplet at 0.84 accounted for six protons of two terminal methyl groups. Two triplets appeared at 4.13 and 2.26 were assigned to methylene groups attached to ester oxygen ($-COOCH_2-$) and ester carboxyl group ($-CH_2-COO-$) respectively. A complex multiplet for one proton was observed at 3.50 and assigned for hydroxyl group. A broad signal was observed at 1.23 for the rest of the forty protons of the methylene groups. On the basis of above discussion, the structure of compound B was established as 6-hydroxypentacosylpentanoate.

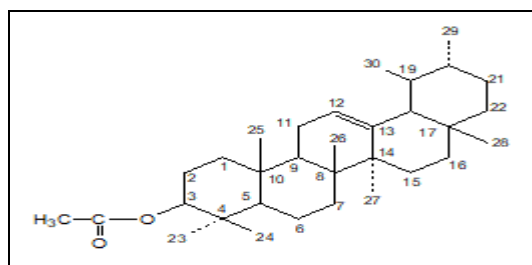


COMPOUND B

Compound C: The triterpenoid nature of compound C was confirmed by tests with Liebermann-Burchard and Noller's reagent. A positive test with TNM indicated the unsaturated nature of the compound. In the mass spectrum of the compound, the molecular ion peak was observed at m/z 468 (M^+). Other important peaks were observed at m/z 453, 409, 218, 203, 189, 135, etc. The 1H NMR spectrum of the compound accounted for fifty-two protons and ^{13}C NMR spectrum showed thirty-two signals for the carbon atoms. With the help of the above observations, the molecular formula of the compound C was established as $C_{33}H_{52}O_2$. In the IR spectrum (KBr, cm^{-1}) a characteristic absorption observed at 1739 suggested the presence of an acetyl group. The presence of C-O stretching was established for the absorption in 1050.

The absorption at 1650 accounted for C=C stretching. The presence of gem dimethyl deformation was assigned by the absorptions at 1385 and 1375. The 1H NMR spectrum (δ ppm, $CDCl_3$) showed six singlets at 0.80, 0.86, 0.78, 0.97, 1.03 and 1.07 corresponding to the protons of the methyl groups present at C-25, C-26, C-24, C-27, C-23 and C-28 positions respectively. The absorption signal for the protons of rest of the two methyl groups present at C-29 and C-30 positions appeared as two doublets at 0.95 and 0.92 respectively. A triplet observed at 4.55 accounted for the one proton at C-3 position. A characteristic singlet was observed at 2.04 for three protons due to presence of an acetyl group at C-3 position. A complicated pattern observed between 1.28-1.93 was assigned for the remaining twenty three protons. Absorption at 5.15 has been assigned to olefinic proton attached at C-12 position. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) displayed the absorptions at 28.14, 16.85, 15.06, 17.15, 24.05, 28.10, 22.95 and 20.85 corresponding to methyl groups attached to C-23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. Two signals at 123.55 and 138.85 were assigned to two olefinic carbons *i.e.* C-12 and C-13 respectively.

The carbon atom of the methyl group of an acetyl group (CH_3-COO-) appeared at 23.05 and the oxygen-bearing carbon atom of the acetyl group (CH_3COO-) appeared at 171.25. Other important absorptions were observed at 38.55 (C-1), 23.01 (C-2), 78.95 (C-3), 37.25 (C-4), 55.55 (C-5), 18.04 (C-6), 23.25 (C-7), 40.52 (C-8), 47.95 (C-9), 37.00 (C-10) and 17.85 (C-11). The values of other carbons were observed at 42.00 (C-14), 28.24 (C-15), 37.85 (C-16), 33.08 (C-17), 59.45 (C-18), 40.46 (C-19), 39.00 (C-20), 31.51 (C-21), 41.65 (C-22) and their assignments have been given in parentheses. On the basis of the above observations and comparing these values with the reported data compound, C was identified as α -myrin acetate.



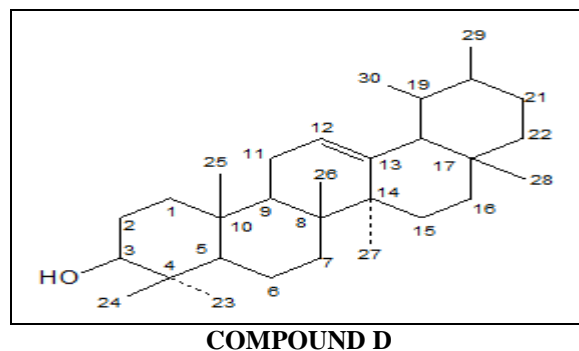
COMPOUND C

Compound D: It gave a positive test with Liebermann-Burchard and Noller's reagents suggesting the triterpenoid nature of the compound D. Positive test with TNM indicated that the compound should be unsaturated triterpene. In the mass spectrum, the presence of molecular ion peak at m/z 426 (M^+) suggested the molecular formula of the compound as $C_{30}H_{50}O$. The IR spectrum (KBr, cm^{-1}) of the compound D showed a broad absorption signal at 3250-3200 which confirmed the presence of hydroxyl group. A characteristic absorption observed in 1655 was due to C=C stretching. The presence of gem dimethyl group [$>C(CH_3)_2$] deformation was indicated by absorptions at 1385 and 1370. Strong absorption at 1060 showed the presence of C-O stretching.

The 1H NMR spectrum (δ ppm, $CDCl_3$) of the compound D showed the presence of six sharp singlets at 0.82, 0.79, 0.95, 0.76, 1.06 and 1.04 corresponding to the protons of the methyl groups present at C-25, C-26, C-23, C-24, C-27, and C-28 positions respectively whereas the presence of two doublets at 0.88 and 0.86 were assigned to the protons of methyl groups at C-29 and C-30 positions respectively. An absorption at 5.06 has been assigned to the olefinic proton at the C-12 position. A triplet observed at 4.43 confirmed the presence of a proton at the C-3 position. Absorptions corresponding to the remaining twenty-four protons were observed between 1.25-2.10. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) displayed the absorptions at 16.52, 16.84, 15.61, 21.45, 23.44, 28.36, 28.57 and 23.67 corresponding to C-24, C-26, C-25, C-30, C-29, C-28 and C-27 respectively. The absorption at 79.23 accounted for the carbon atom at the C-3 position. The two olefinic carbons at C-12 and C-13 showed absorptions at 124.56 and 139.87 respectively.

Other characteristic absorptions were assigned to remaining carbon atoms as 18.55 (C-6), 17.63 (C-11), 23.72 (C-2), 31.50 (C-21), 38.43 (C-1), 40.05 (C-19), 23.67 (C-7), 37.32 (C-10), 33.23 (C-17), 28.42 (C-15), 39.22 (C-20), 41.34 (C-22), 37.54 (C-4), 37.12 (C-16), 40.48 (C-8), 42.36 (C-14), 59.37 (C-18), 48.20 (C-9) and 55.61 (C-5). The assignments of the positions of carbon atom have been given in the parentheses. The above spectral data were in good agreement with those reported for α -amyrin in literature.

Based on these observations the structure of the compound D was confirmed as α -amyrin.

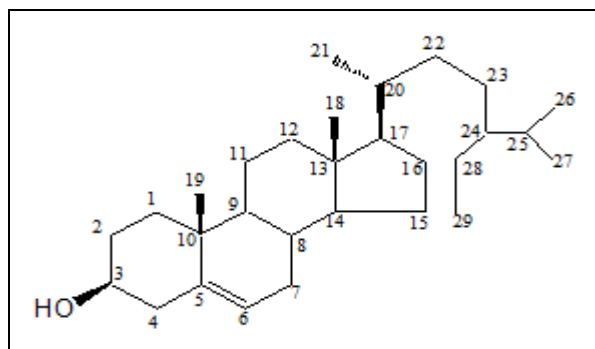


Compound E: In the mass spectrum of the compound E molecular ion peak was observed at m/z 414 (M^+). Other significant ions were observed at m/z 397, 383, 369, 255, etc. On the basis of the mass spectrum, the molecular formula of the compound E was established as $C_{29}H_{50}O$. The unsaturated nature of compound E was suggested by the positive TNM test. The IR spectrum (KBr, cm^{-1}) displayed strong absorption at 3500-3440 which indicated the presence of hydroxyl group. The absorption at 1050 was assigned for C-O stretching whereas the absorption at 1580 accounted for the presence of olefinic ($>C=C<$) group. The 1H NMR spectrum (δ ppm, $CDCl_3$) of compound E showed strong absorption at 0.83 and 0.94 as two doublets which confirmed the presence of protons of the two methyl groups at C-26 and C-27 positions respectively. The presence of the tertiary methyl group at C-18 position was confirmed by a singlet observed at 0.65 for three protons. A doublet was observed at 1.24 for three protons corresponding to the methyl group present at the C-21 position. Whereas a triplet observed at 0.96 accounted for the three protons of a methyl group at C-29 position. The methyl group present at C-19 position appeared at 0.98 as a singlet.

The presence of methine proton at the C-25 position was indicated by absorption at 1.83 as a multiplet. The olefinic proton present at C-6 was assigned as a triplet at 5.30 with coupling constant $J = 2.8$ Hz. A double doublet signal was observed at 2.14 for methylene protons at C-7. A multiplet observed at 3.52 was assigned for a methine proton at C-3 position. The chemical shift and coupling constant $J = 5.60$ Hz of methine proton supported the β -orientation of hydroxyl group at the C-3 position.

In the ^{13}C NMR spectrum (δ ppm, CDCl_3) the olefinic nature of the C-5 and C-6 carbon atoms was confirmed by the absorption at 140.02 and 122.15 respectively. An absorption at 72.00 confirmed the presence of hydroxyl group at C-3 position. Other characteristic absorptions were observed at 31.32 (C-1), 32.00 (C-2), 42.22 (C-4), 32.03 (C-7), 46.14 (C-8), 49.80 (C-9), 36.14 (C-10), 29.95 (C-11), 28.22 (C-12), 42.36 (C-13), 57.00 (C-14), 24.34 (C-15), 40.14 (C-16), 56.25 (C-17), 36.23 (C-20), 16.16 (C-22), 24.65 (C-23), 39.92 (C-24), 36.01 (C-25), 32.20 (C-28), 12.00 (C-18), 19.54 (C-19), 19.53 (C-21), 23.42 (C-26), 23.44 (C-27) and 29.47 (C-29).

The above spectral data were found to be in good correlation with those reported in literature for β -sitosterol. On the basis of the above spectral evidences the compound E was characterized as β -sitosterol.



COMPOUND E

CONCLUSION: As the various parts of the title plant are used in the treatment of the different medicinal conditions in traditional medicines and it shows promising medicinal properties. So, the compounds isolated can have future importance in drug development.

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CONFLICTS OF INTEREST: Authors declare no conflicts of interest.

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