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ISOLATION AND CHARACTERIZATION OF PHYTOSTEROID AND TRITERPENODS FROM LEAVES OF ABUTILON CRISPUM

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ABSTRACT

Natural product have served as an important sources of drugs since ancient time and about half of the useful drugs today are derived from natural source. The study of natural products has advantage over synthetic drugs. Most of the phytochemical derived from natural source are extremely useful as lead structures for synthetic modifications and optimization of bioactivity. The aim of the study is to identify and characterize the bioactive compounds from leaves of *Abutilon crispum.* For isolation of the compounds, the dried leaves powder *Abutilon crispum* was subjected to extraction with chloroform and subject to column chromatography with different solvent fractions. Three compounds were isolated from the chloroform extract and purified. These compounds were eluted with benzene and petroleum ether. The structures of these compounds were characterized by using IR, NMR and Mass spectrometry. These compounds were isolated from this plant for the first time.

KEYWORDS: *Abutilon crispum, Isolation, IR, NMR.*

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INTRODUCTON

Abutilon crispum belonging to family Malvaceae is a trailing perennial, weak, shrub. This plant is commonly distributed in the shady undergrowth forest on hilly slopes found throughout India. It is known as Errabenda in Telugu[1]. The plant finds its application in the traditional system of medicine. In India, this plant is used in the treatment of Asthma in Rayalaseema Region of Andhra Pradesh and it is also used for the treatment of Piles by the Kondadora Tribe of Northern Andhra Pradesh[2]. Its leaves are used for treating asthma, piles, ulcers, cough, jaundice and diabetics by tribal people of Andhra Pradesh and its fruits are used in the treatment of piles in Tamilanadu. The plant is used as a leafy vegetable by the Paliyar tribes of Pachalur in Dindigul district of Tamilnadu [3-6].

MATERIALS AND METHODS

Plant Collection and Preparation

The fresh leaves of *Abutilon crispum* collected from Pakala (Warangal) in the month of August were subjected to be authenticated by Prof. V. S. Raju, Department of Botany, Kakatiya University and Warangal,voucher specimen (MRM/03/2012) was deposited in the College of Pharmaceutical Sciences, Andhra University; Visakhapatnam. After confirmed authenticity and leaves were manually separated. The plant material was washed with water to remove soil, mud, debris and other adhering materials and dried thoroughly in air under shade at room temperature. Coarse prepared powdered drug was passed through sieve no.40 and stored in air tight containers.

Extraction and Isolation

About (1kg) of powdered leaves of *Abutilon crispum* was extracted with chloroform using Soxhlet apparatus. The extract was concentrated with rotary evaporator. the chloroform extract was stored in screw cap vial at 4°C until further use. The chloroform extract upon concentration under reduced pressure left a dark gummy residue (15g). The extract showed positive Liebermann-Burchard reaction (pink-Blue-Green) and negative ferric chloride, Shinoda tests, indicates the presents of steroidal compounds.The 15g of chloroform extract was admixed with 15g silicagel (60/120 meshes) to get uniform mixing. 150 g of silica gel (70/325 meshes). The column was kept aside for 1 hr and allowed for close packing. Admixture was then added at the top of the stationary phase and separating of the compounds as started by eluting with various solvent such as petroleum ether, benzene and petroleum ether with different ratios with increasing of polarity. All the column fractions were collected separately and concentrated.

Spectroscopic characterization

Different spectroscopic methods were used to elucidate the structure of compounds. Among the spectroscopic techniques IR,^{1}H Nuclear Magnetic Renounce and ^{13}C NMR as carried out. The infra red spectrum as recorded on Perkin Elmer FTIR88, ^{1}HNMR spectra were recorded on a 90MHz, Jeol JNM EX-90 FT NMR, 300 MHz Bruker FT DRX-300. The ^{1}HNMR was recorded using $CDCl_3$, as solvent with Tetramethyl silane (TMS) as an internal standard.

RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the chloroform extract of the leaves of *Abutilon crispum* provided a total of three compounds (compound 1, 2, and 3). The structures of which were determined by extensive IR,^{1}HNMR and ^{13}CNMR spectral analysis as well as by comparison of their spectral data with previously reported values.

Characterization of compound -1

It crystallized as needles from petroleum ether, m.p. 136-138°C. It showed colour reactions in L.B. test, characteristic of sterols (play of colours) and gave a single spot on silver nitrate impregnated TLC. The IR absorption spectrum showed absorption peaks at 3373cm-1(O-H stretching.); 2940cm-1and 2867cm-1 (aliphatic C-H stretching); 1641cm-1 (C=C absorption peak); other absorption peaks includes 1457cm-1 (CH2); 1381cm-1 (OH def), 1038 cm-1(cycloalkane) and 881.6 cm-1,The 1HNMR spectrum of the compound (1) (Fig-1) has revealed a one proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroid nucleus. The typical H-6 of the steroidal skeleton 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 1HNMR spectrum showed a doublet at δ 0.96 with coupling constant 6.5 Hz for a methyl group at C-20. The spectrum also showed three protons triplet at δ 0.89 which could be assigned to the primary methyl group at C-28 and 13CNMR has given signal at 150.98,145.2 (C-5), 139.8 (C-22), 121.7, 118.89(C-6), 79.03 (C-3), 55.3(C-14), 55.18(C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1(C-12),39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.18 (C-1), 37.12 (C-10), 36.3 (C-8), 35.59(C-20), 34.29 (C-22), 34.24 (C-7), 32.66 (C-8), 29.86 (C-25), 29.71 (C-16), 28.41 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11,26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29). On this basis, compound-1 was characterized as β-Sitosterol, the identity of which was confirmed by comparison of the spectral data with previously reported values 7

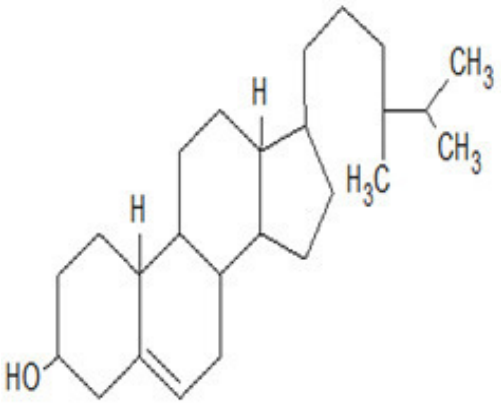

Figure1
Chemical structure of β-Sitosterol

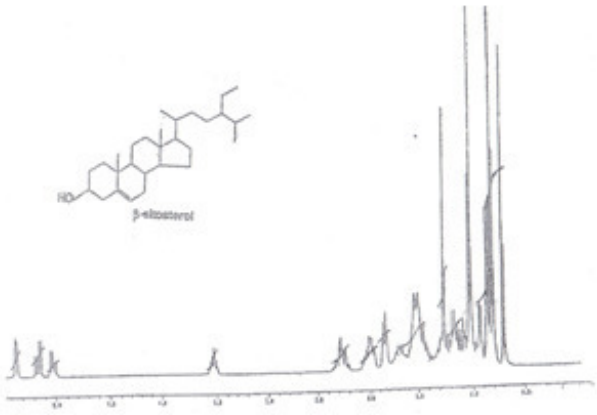

Figure 1.1
^{1}H NMR of β-Sitosterol

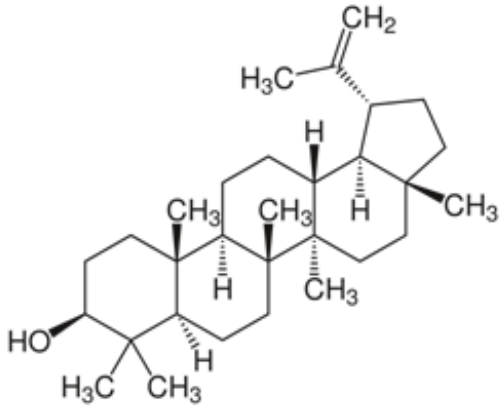

Figure 2
Chemical structure of Lupeol

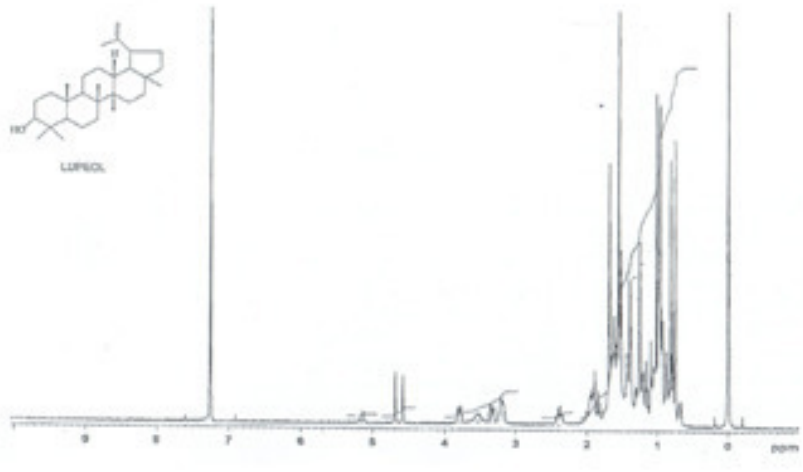

Figure 2.1
^{1}H NMR of Lupeol

Characterization of compound -2

It crystallized from petroleum ether-Benzene as needles, m.p. 212-213°C, It gave pink colour in L.B. reaction and yellow colour with tetranitromethane. In TLC, it corresponded to Lupeol and the identity was confirmed by comparison with an authentic sample (mmp and Co-TLC). The compound showed IR peaks corresponding to hydroxyl (3410, 1042 cm-1) and vinylidine group (1640 and 881 cm^{-1}), 2940, 2870, 1640 (>=CH2), 1453, 1380 (-C-Me2), 1042 (OH), 881 (>=CH2),The ^{1}HNMR spectrum of the compound (2) (Fig-2) that 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 double quartet with coupling constants 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 attributed to a methine group. The resonance signals at δ0.75, 0.77, 0.80, 0.92, 0.94 and 1.02 exhibited six tertiary methyl singlets and ^{13}C NMR (CDCl3, 100 MHz) δ: 38.77 (C – 1), 27.46 (C – 2), 79.02 (C – 3), 38.87 (C – 4), 55.31 (C – 5), 18.33 (C – 6), 34.42 (C – 7), 40.84 (C – 8), 50.45 (C – 9), 37.18 (C – 10), 20.94 (C – 11), 25.15 (C – 12), 38.07 (C – 13), 42.84 (C – 14), 27.46 (C – 15), 35.59 (C – 16), 43.01 (C – 17), 48.72 (C – 18), 48.32 (C – 19), 150.99 (C – 20), 29.86 (C – 21), 40.01 (C – 22), 27.99 (C – 23), 15.37 (C – 24), 16.12 (C – 25), 15.98 (C – 26), 14.55 (C – 27), 18.01 (C – 28), 109.32 (C – 29), 19.31 (C – 30). These data were in close agreement with those reported for a typical pentacyclic triterpenoid Lupeol and further confirmed the identity of compound- 2 as Lupeol[8].

Characterization of compound -3

It crystallized from acetone as colourless plates, m.p. 199-200°C and showed colour reactions characteristics for triterpenes. In TLC, it corresponded to β-Amyrin and the identity was confirmed by comparison with an authentic sample (mmp and Co-TLC). The IR absorption spectrum showed absorption peaks at 3373cm-1 (O-H stretching.); 2940 cm-1and 2867cm-1 (aliphatic C-Hstretching); 1641 cm-1 (C=C absorption peak); other absorption peaks includes 1457cm-1 (CH2); 1381 cm-1 (OH def), 1038.7cm- (cycloalkane) and 881 cm-1,The ^{1}HNMR spectral data showed the presence of a downfield methine proton at δ 3.3 (1H, m) characteristic of H-18 of oleanene derivatives, an olefinic proton signal at δ 5.4 (br, s, H-12). The spectra also ^{1}HNMR showed the presence of eight tertiary methyls in the region δ 1.25-0.78 indicative of oleanene skeleton in compound and^{13}C NMR δ 39.59 (C-1), 27.93 (C-2), 80.8 (C-3), 39.59 (C-4), 55.24 (C-5), 18.19 (C-6), 33.73 (C-7), 38.37 (C-8), 47.4 (C-9), 35.55 (C-10), 23.59 (C-11), 121.62 (C-12), 145.20 (C-13), 42.05 (C-14), 28.73 (C-15),27.93 (C-16), 32.85 (C-17), 59.0 (C-18), 40.01 (C-19), 41.52 (C-20), 31.23 (C-21), 42.01 (C-22), 29.69 (C-23), 15.72 (C-24), 15.72 (C-25), 16.84 (C-26), 23.59 (C-27), 28.73 (C-28), 17.50 (C-29),21.39 (C-30). These data were in close agreement with those reported for a typical pentacyclic triterpenoid and further confirmed the identity of compound- 3 as β- Amyrin.- 3.

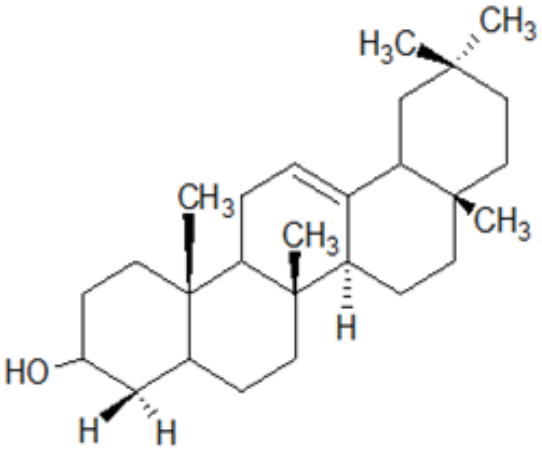

Figure 3
Chemical structure of β- Amyrin

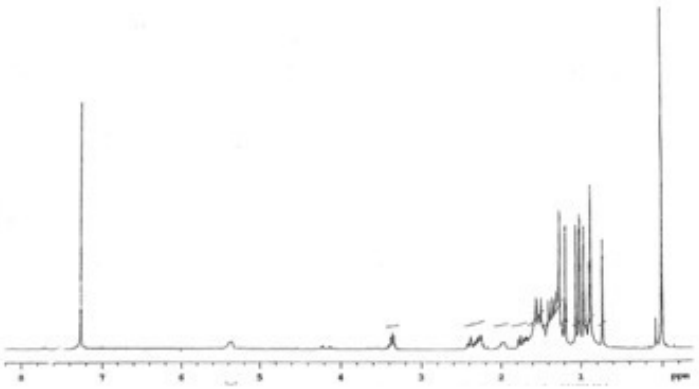

Figure 3.1
^{1}H NMR of β- Amyrin

CONCLUSION

Chemical examination of the leaves of *Abutilon crispum* on conventional extraction and a sequential chromatography afforded three compounds. From the chloroform extract three compounds were separated and identified as β-Sitosterol, Lupeol and β-Amyrin. All the compounds were characterized by physical, chemical and spectral studies using IR, ^{1}H NMR, and ^{13}C NMR.This is the first report of occurrence of this compound in this plant. Therefore future biological investigations are needed for these isolated compounds.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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