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Full Length Research Paper

Isolation and characterization of a steroidal compound from the hexane extract of the leaves of *Artabotrys odoratissimus*

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Artabotrys odoratissimus **has been investigated by many workers for its constituents. Previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthones and triterpenoids. So far, not many studies have been carried out on this genus but there are some reports on this plant. Our recent study on the hexane extract of the leaves of *A. odoratissimus*, have led to the isolation of steroidal compound. The structure of the compound has been established by modern spectroscopic techniques such as IR, ^{1}H-NMR, ^{13}C-NMR and mass-spectroscopy and identified as tetracontan-15-one.**

Key words: *Artabotrys odoratissimus*, medicinal plant, tetracontan-15-one.

INTRODUCTION

Artabotrys odoratissimus, commonly known as KantiliChampa, is an ornamental shrub. Leaves are oblong, lanceolate, glabrus, shining acute at the base, petioles are 6 to 10 mm long. The size of the leaves are up to 18 by 3.8 to 5.0 cm. It is available in Bangladesh and India (Chopra et al., 1956). Ayurvedic and Yunani doctors use the leaves, flowers as a remedy for cholera, vomiting, thirst and headache. Volatile oils from the leaves show antifungal and antimicrobial activity. The antifertility activity of *A. odoratissimus*, plant has been reported in albino rats (Chakarabarti et al., 1968). The fruit extracts showed cardiac stimulatory effects on some animals and cardiac depressant effects on others (Trivedi et al., 1971). So far, not many studies have been carried out on this genus but previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthones and triterpenoids (Connoly et al., 1994; Haider et al., 1991; Perold et al., 1978; Bheemasankara et al., 1984; Sharma et al., 2002; Singh et al., 2009; Chakabati et al., 1968). The compounds so far isolated from the leaves of *A. odoratissimus* are pentadecyl-7- hydroxy-dodecanote,pentadecyltritriacontanonte,4,5-epoxy-26-ol-dopentacontane,β- sitosterol, Aplysterol, Nonacosanylhexaconsanoate, Pentatetraacont-19-ol,triacont-2- ol, Dotriacont-7-ene, Octacose-7-ene,1-hydroxy-2,5-dimethyl-9, 10-anthraquinone,1,4,5-trihydroxy9, 10anthraquinone, 13hydroxynonacosane, Nonanoicacid, Methylphenylpropanoate, Decanoicacid,

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Diethylphthalate, Dibutylphthalate, 2-amino-3-ethylbiphenyl,5-methyl-9- phenylnonane-3-ol,1-phenylundecane,2,5-dimethyl-1-phenylheptane1-one, hexadeca-2,7,11- triene, 1-isopropyl-4,6-dimethylnaphthalene, 1-phenyldeca-1-one,1-phenylundecan-1-one, 5- (2-butylphenyl) pent-3-en-2-ol,2,5-dimethyltetra decanhydrophenenthrene. Our recent study on the leaf extracts have led to the isolation of steroidal compound. In this paper, isolation and structural elucidation of the isolated compound from the hexane extract of the leaves of *A. odoratissimus* is described. The isolated compound identified by spectral data, has not been reported before.

EXPERIMENTAL: GENERAL

Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure in a Buchii rotary evaporator. IR spectra were recorded (KBr discs) on a Shimadzu UV-168A Spectrophotometer, validation (Vmax in cm^{-1}). ^{1}H-NMR were recorded on a Bruker R-32 (300 MHz) instrument in $CDCl_3$ and DMSO-d6 with TMS as an internal standard (Chemical Shifts in δ, ppm). All solvents used were of analytical grade. Thin-layer chromatography (TLC) was performed using Silica gel GF254.

Plant materials

The leaves of *A. odoratissimus* were collected from the gardens of Ujjain city and university campus and were identified by the authorities of IEMPS, Vikram University Ujjain (M.P) India.

Extraction of the Compound D1

Dried leaves of the plant (5 kg) were milled into powder and then extracted with hexane (8 L) in a Soxhlet extractor for 36 h. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The hexane extract (50 g) was suspended on water and extracted successively with hexane, hexane: Benzene, Benzene: Ethyl acetate and Ethyl acetate:Chloroform to yield hexane (15 g), Hexane: Benzene (5 g), Benzene:Ethylacetate (12 g), Ethylacetate:Chloroform (3.2 g), soluble fractions respectively. The hexane (15 g) soluble fraction was subjected to column chromatography was eluted initially with hexane, hexane: Benzene followed by the mixture of Benzene with increasing amount of ethyl acetate and finally with methanol. These elutes were collected in a series of test tubes with 15 ml in each fraction. Each fraction was examined by TLC. Based on the similar TLC behavior, these elutes were combined to yield D1, D2, D3, D4, D5, D6, D7, D8, D9 and D10. The fractions D1-D3 (that is, hexane fractions) were concentrated and allowed to stand at room temperature when a circular white amorphous crystal was obtained and this was marked as compound D1. The amorphous crystal was washed with methanol successively. The crystal dissolved in chloroform, applied in TLC micro-glass slide, and viewed in an iodine chamber were two different spots were detected. Therefore, the compound was needed further purification and dissolving in the mixture of $CHCl_3$ and methanol (10:1) and kept at room temperature. After 24 h a white amorphous crystal was precipitated out at the bottom of the conical flask. The crystallization process was repeated three times and finally white crystal (80 mg) were obtained.

Compound D1

Finally the Compound D1 was crystallized from Benzene: ETOAC (3:7v/v) to give white crystals (90 mg), M.P. 285.
IR. λ (KBr):2919,2850,1735,1636,1541,1463,1261,1074,801-668 cm^{-1}.
$2CH_3$, J=6.9Hz), 2.35(t,4H, CH_2COCH_2, j=7.5Hz),1.56(s,4H,2-βCH_2),1.26(s,66H,-$33CH_2$).
^{13}CNMR (75 MHz, $CDCl_3$, TMS): 15.5, 22.7-29.9, 31.9, 35.5, 173.8 ppm.
EIMS(m/z,rel,int):M+576(5.85),533(4.05),504(4.95),476(9.9),462(12.16),435(11.26),406(7.20),378(14.86),337(27.77),308(19.81),280(19.80),253(24.54),240(28.37),238(247.77),182(27.47),141(34.68),126(34.68),84(34.68),58(100),43(27.92),28(24.77).

RESULTS AND DISCUSSION

The TLC examination of the isolated compound from the hexane extract of the leaves of *Artabotrys odoratissimus* upon exposure to iodine vapour showed a bright single spot. The compound was obtained as white crystals (m.p=285°C). HRMS of compound D1 exhibited molecular ion at m/z 576, which is consistent with the molecular formula $C_{40}H_{80}O$. It was readily soluble in $CHCl_3$. IR spectrum showed bands at 2919, 2850,1735, 1541, 1463, 1074, 801, 730 to 720 cm^{-1} suggesting aliphatic ketonic nature of the compound(Mehta et al., 1999; Bellamy, 1962).

The ^{1}H NMR spectrum showed triplet at δ 0.86 for six protons of terminal methyl groups. A triplet at δ 2.35 for alpha methylene (CH_2OCH_2) protons to the carbonyl group. A broad singlet at δ 1.56 for beta methylene protons. A broad singlet at δ 1.26 assigned to rest of the methylene protons (Falch et al., 2004; Fauhl et al., 2000; Guillen and Ruiz, 2005; Guillen and Ruiz, 2003; Panico et al., 1994; Gunstone et al., 1975; Lie Ken Jie et al., 2003). C^{13} NMR spectrum value were consistent with the proposed structure, it showed a peak at δ 173.8 for carbonyl carbon. The peaks at δ 35.5 and 31.9 attributed to the methylene carbons attached at alpha and beta positions of the carbonyl group. A bunch of peaks at δ 29.9-22.7 corresponds to the remaining methylene carbons. Peak at δ 15.5 correspond to the methyl carbons.

The EIMS showed the molecular ion peak at m/z 567 suggesting its molecular formula as $C_{40}H_{80}O$. The mass fragmentation was characteristic of long chain hydrocarbon. The separation of most of the peaks by 14 and 28 mass units and appearance of CnH_2n+1 and CnH_2n-1 ion series confirmed its long chain aliphatic nature. The fragment at m/z 240 formed by the Mc Lafferty rearrangement indicated the position of carbonyl group. Other abundant fragments at m/z504, 476, 462, 450, 435, 378, 182, 141 and 58 were characteristic of the long chain aliphatic ketone (Mehta et al., 1999; Gowarikar et al., 1997; Bellamy, 1962; Manorangani et al., 1999; Silverstein et al., 1984; Christie et al., 1993; Ali et al., 2001; Dobson and Sebedio, 1999; McLafferty, 1973).

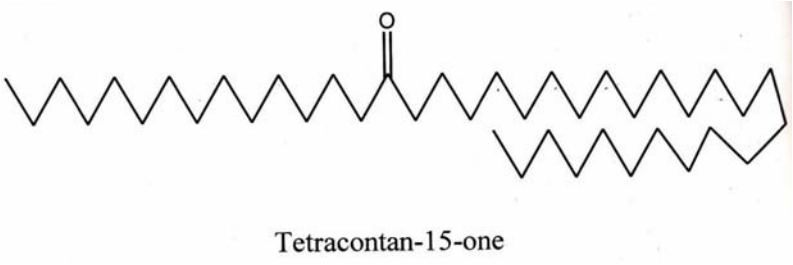


Figure 1. Tetracontan-15-one.

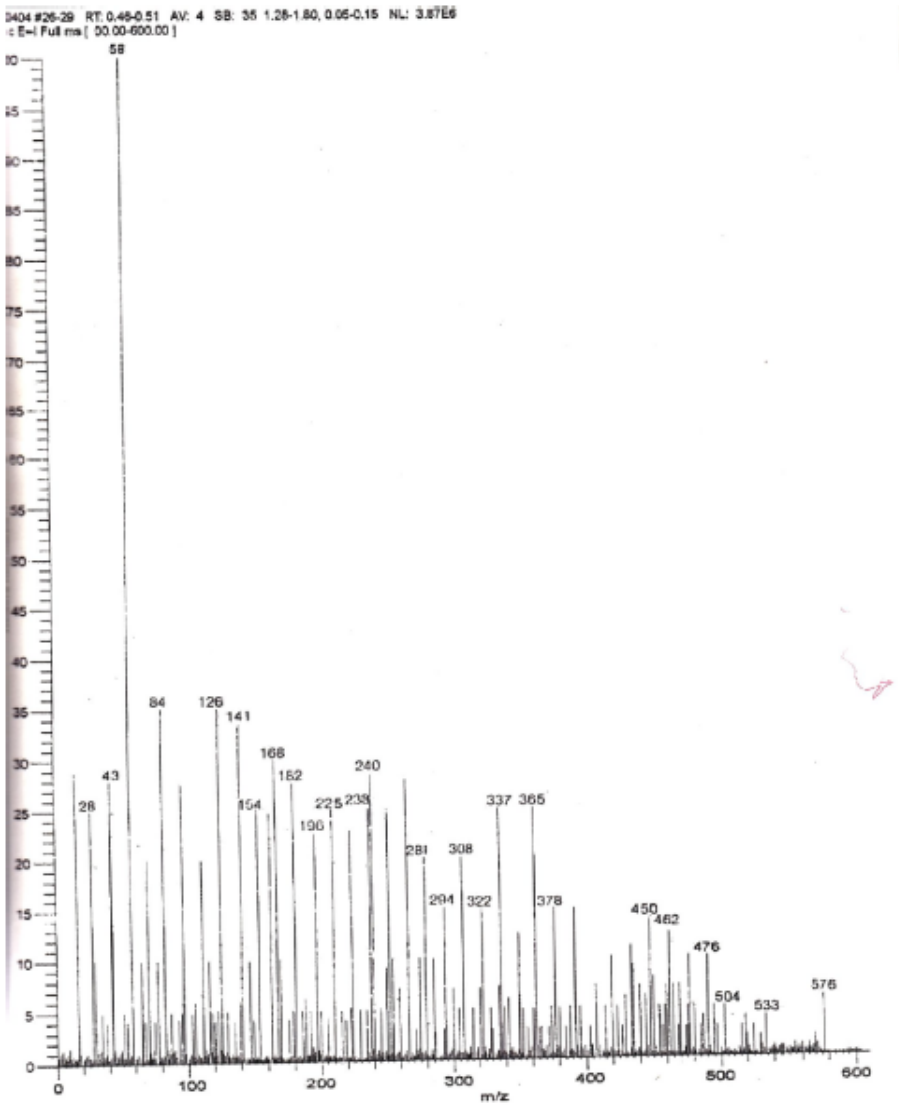


Figure 2. EI-MS spectrum of D1.

Thus the IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis with physical properties established the identity of Compound D1 as tetracontan-15-One (Figures 1 to 5).

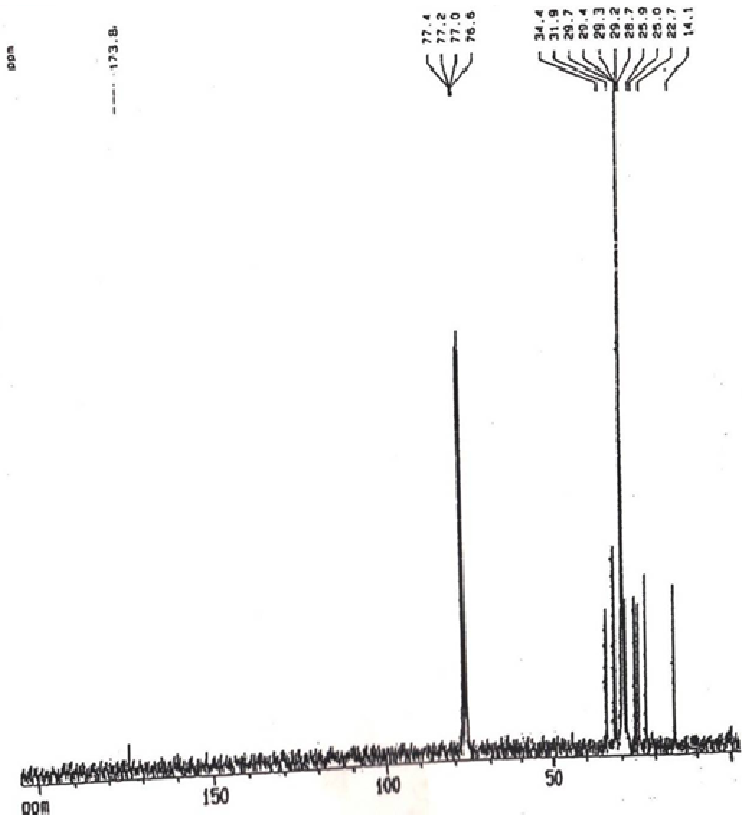


Figure 3. ^{13}CNMR spectrum of D1.

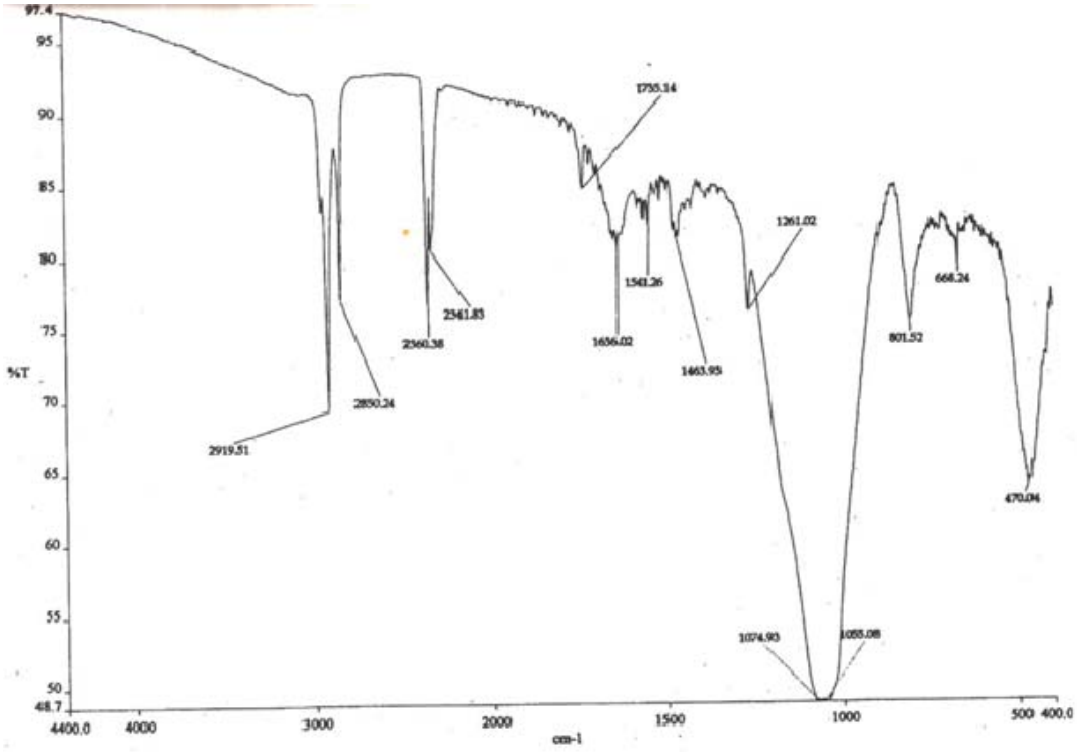


Figure 4. IR spectrum of D1.

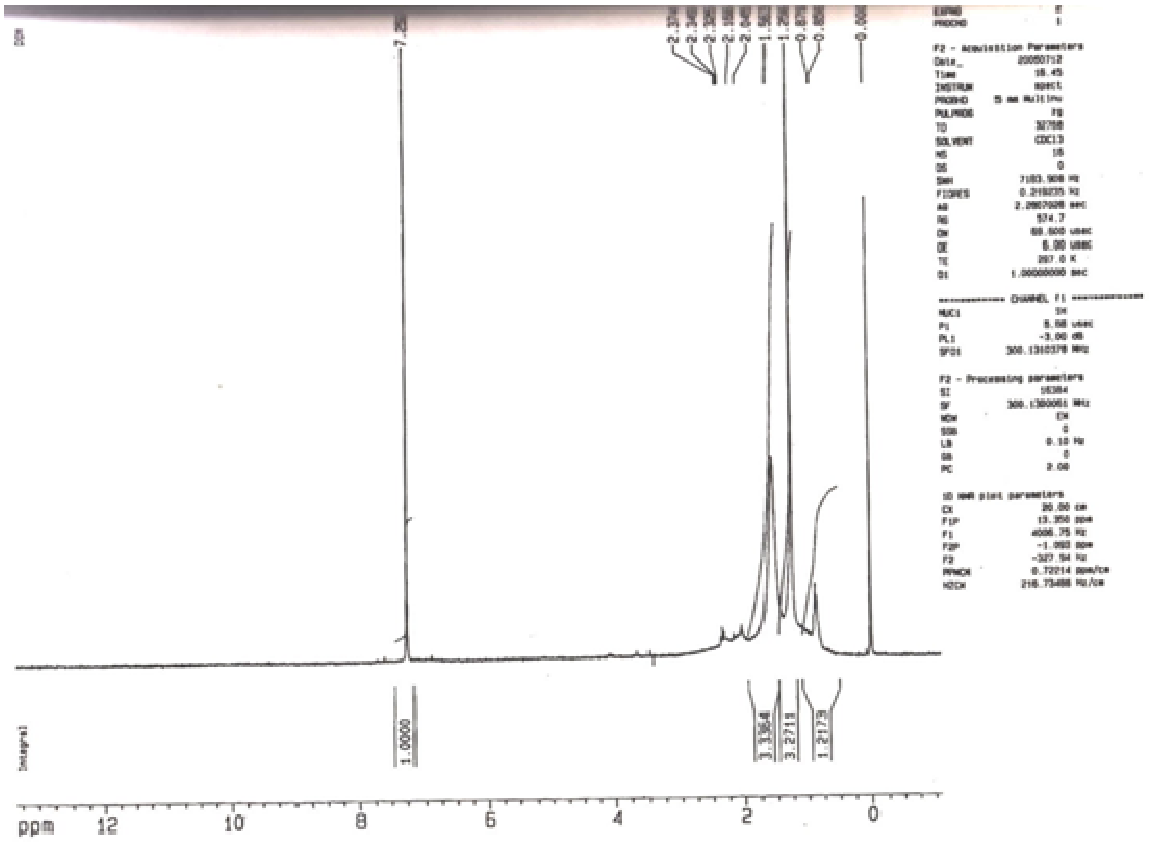


Figure 5. 1H NMR spectrum of D1.

Conclusion

The results of the present investigation showed the occurrence of Tetracontan-15-onetype compound in plant kingdom. The title Compound was isolated from this plant for the first time.

Conflict of Interest

The authors have not declared any conflict of interest.

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