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Phytochemical study on the benzene:acetone extract of the leaves of *Artabotrys odoratissimus*

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***Artabotrys odoratissimus* has been investigated by many workers for its constituents. 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-trihydroxy-9,10-anthraquinone, 13-hydroxynonacosane, Nonanoicacid, Methylphenyl propanoate, Decanoicacid, Diethylphthalate, Dibutylphthalate, 2-amino-3-ethylbipheny-l, 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-dimethyltetradecanhydro phenenthrene So far, not many studies have been carried out on this genus but there are some reports on this plant. Previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthones and triterpenoids. Our recent study on the benzene:acetone 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 Infrared Spectrometry (IR), Hydrogen-Nuclear Magnetic Resonance Spectrometry (^{1}H-NMR), Carbon-Nuclear Magnetic Resonance Spectrometry (^{13}C-NMR) and Mass-Spectroscopy and identified as 2β- hydroxy- Stigmasta-4- en-3, 6- dione- Methoxy ester.**

Key words: *Artabotrys odoratissimus*, medicinal plant, new compound, spectral analysis.

INTRODUCTION

Artabotrys odoratissimus, commonly known as Kantili Champa, is an ornamental shrub distributed throughout the country. Leaves are oblong, lanceolate, glabrus, shining acute at the base, petioles are 6 to 10 mm long. The size of the leaves is up to 18 by 3.8 to 5.0 cm. Flowers are acrid, bitter and yellowish white in colour. It is available in Bangladesh and India (Chopra et al., 1956). Ayurvedic and Yunani doctors use the leaves and flowers as a remedy for cholera, vomiting, thirst, headache and 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). Previous Phytochemical studies have revealed this genus to be rich in secondary metabolites including, alkaloids, flavenoids,

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Table 1. Column chromatography processing of benzene:acetone extract of *A. odoratissimus* (leaves).

Fraction No.	Eluent	Ratio of eluent (v/v)	Volume collected (ml)	TLC spots	Yield (gram)
1	Hexane	-	2500	3 Spots with streak	11
2	Hexane:benzene	1:1	3000	Dark streak	7
3	Hexane:benzene	2:3	2000	Unresolved streak	8
4	Hexane:benzene	1:4	5000	Single spot	13
5	Benzene:ETOAC	3:1	3000	2 Spots with streak	18
6	Benzene:ETOAC	1:1	3000	Single spot with streak	30
7	Hexane:MeOH	2:1	3000	Single spot with streak	5
8	MeOH	-	3000	Unresolved streak	3.2

Weight of Silica Gel = 900 g; Weight of extract = 100 g.

Table 2. Re-column chromatography processing of fraction No.6 (From Table 1).

Fraction No.	Eluent	Ratio of eluent (v/v)	Volume collected (ml)	TLC spots	Yield (mg)
1	Hexane:benzene	1:1	1000	Unresolved streak	19
2	Benzene:EtOAC	3:2	3000	2 Spots with streak	11.2
3	EtOAC:$CHCl_3$	3:1	3000	Unresolved dark streak	13
4	EtOAC:$CHCl_3$	2:1	5000	Compound S1	60.2

Weight of Silica gel = 200 g; Weight of extract = 30 g.

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). In this paper, we have described the isolation and structural elucidation of the isolated compound from the benzene: acetone extract of the leaves of Artabotrys odoratissimus. The isolated compound is identified by its Spectral data and has not been reported before.

MATERIALS AND METHODS

General

Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure on a Buchii rotary evaporator. Infrared (IR) spectra were recorded (KBr discs) on a Shimadzu UV-168A Spectrophotometer, validation (Vmax in cm^{-1}). Hydrogen-Nuclear Magnetic Resonance Spectrometry (^{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 delta, 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).

Extraction of the Compound S1

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 hexane extract was evaporated in a rotator evaporator and dried by vacuum pump. The hexane extract (200 g) was extracted successively with hexane, benzene, benzene:acetone, benzene:EtOAC and ethanol to yield hexane (17.5 g), benzene (14 gm), benzene:acetone (100 gm), benzene:EtOAC (11 g) and ethanol (2.3 g) soluble fractions, respectively. The benzene:acetone (100 g) soluble fraction on TLC examination showed several spots. To isolate different compounds, the benzene:acetone extract (100 g) was subjected to column chromatography using Silica gel as adsorbent. The column was eluted with different solvents in their increasing order of polarity. Various elutes showed different pattern of spots on TLC examination and therefore, were further separated by rechromatography on Silica gel columns and the results are being reported in Tables 1 and 2.

Thus, the EtOAC:$CHCl_3$ (2:1,v/v) fraction revealed the presence of single Compound S1 with clear spot on TLC plate.

RESULTS AND DISCUSSION

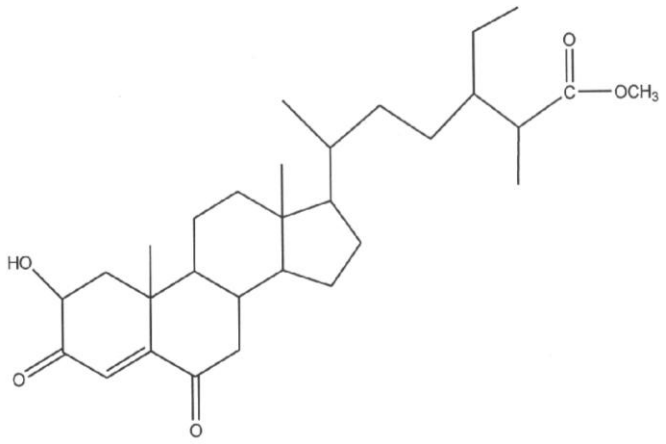
Finally, the Compound S1 was crystallized from benzene:acetone extract to give reddish brown crystals(60.2 mg), m.p. 173 to 179°C; IR (λmax(KBr): 3445, 2919, 2850, 1837, 1733, 1705, 1675, 1640, 1543, 1522, 1461, 1380, 1341, 1250, 1155, 974, 845, 831, 759 and 730 to 720 cm^{-1}; ^{1}H- NMR (300 MHz,δppm, $CDCL_3$, TMS): and ^{13}C- NMR (δppm, $CDCL_3$, 75MHz) (Table 3). FAB-MS(m/z,rel,int):M^+486(6.94), 471(5.56), 469(5.56), 459(5.56), 443(9.72), 441(8.33), 429(69.44), 427(36.11),

Table 3. Crystallization of Compound S1.

Carbon No.	Type of carbon	H1-NMR delta ppm ($CDCL_3$, 300 MHz)	C13- NMR, delta ppm ($CDCL_3$, 75 MHz)
1	CH2	1.49(d, j = 5.1 Hz)	37.52
2	CH	3.51 (m)	72.10
3	C	-	231.0
4	CH	5.82 (S)	122.0
5	C	-	161.7
6	C	-	223.0
7	CH2		34.23
8	CH		31.95
9	CH	1.83	50.42
10	C		36.40
11	CH2	1.09	21.35
12	CH2	1.49, 1.13	42.60
13	C		46.13
14	CH		56.35
15	CH2		24.60
16	CH2	1.95 (m)	28.50
17	C		57.04
18	CH3	0.65 (S)	12.24
19	CH3	1.01 (S)	19.65
20	CH		32.18
21	CH3	0.98	19.04
22	CH2	2.32(d, J = 5.7 Hz)	40.10
23	CH2	1.26	26.40
24	CH	2.23	24.60
25	CH	2.33 (m)	29.50
26	CO		183.4
27	CH3	0.82 (d, j = 7.5Hz)	19.30
28	CH2		23.35
29	CH3	0.95 (t)	12.12
30	OCH3	3.75 brs	64.87

423(11.11), 411(100), 397(43.05), 395(43.05), 383(15.28), 367(9.72), 353(6.94), 327(5.56), 311(6.94), 299(8.33), 287(6.94), 269(15.28), 239(9.72), 227(12.5), 213(15.28), 199(13.89), 185(18.06), 175(19.44), 159(41.67), 145(43.61), 133(40.28), 107(45.8), 105(48.61), 95(50.0).

The TLC examination of the isolated compound from benzene:acetone extract of the leaves of *A. odoratissimus* showed a single spot upon exposure to iodine vapour. It was readily soluble in $CHCL_3$. To the best of our knowledge, this compound has not been previously isolated or separated from any other sources. 2β- hydroxy-stigmasta-4-en-3,6-dione-methoxy ester was isolated from this plant for the first time in our laboratory. The IR spectrum showed peaks corresponding to enedione pattern (1837 and 1733 cm^{-1}), the carbonyl group (1718 cm^{-1}), C=C unsaturation and isopropyl group (1639 and 1380 to 1341 cm^{-1}), respectively (Ali, 2001; Dewick, 1997; Song et al., 2004; SAS Institute SAS/STAT user's Guide, 1997; Branco et al., 2001). The peak at 3445 cm^{-1} corresponds to hydroxyl group. The enedione pattern is further confirmed by the absorption at 238 nm in the UV spectrum. The peak at 1705 cm^{-1} showed the presence of $COOCH_3$ group in the molecule (Silverstein et al., 1984). The absorption peaks at 759, 831 and 845 cm^{-1} are characteristic of Δ^4- unsaturated and skeletal vibrations of steroidal moiety (Fujita et al., 1992; Bellamy, 1975). The ^1H spectrum showed the presence of two singlets, integrating three protons each at δH 0.65 and 1.01 were assigned to two angular methyl groups at C-18, C-19 and a doublet at δH 0.98 (J = 6.5Hz) to the methyl protons at C-21. A doublet was assigned to 27- Me at δH 0.82 (J = 7.5 Hz). The singlet at δH 5.32 for one proton revealed the presence of Olefinic proton at C-4 in the ring usually observed in the steroids having Δ^4 un-saturation with enedione (Peres and Nagem,



Scheme 1. 2β-hydroxy-stigmasta-4-en-3,6-dione-methoxy ester.

1997; Chung et al., 2006; Li et al., 1999). A multiplet at δH 3.50 showed the presence of 2α- methine proton (axial) interacting with C-1 equatorial, C-1 axial for hydroxyl group in the Ring A. There is a broad singlet at δH 3.75 for methoxy group at C-30 of methyl ester at C-26. The attachment of this group to C-25 is confirmed by the deshielding of H-25 at δH 2.33 as multiplet. A triplet at δH 0.95 was assigned to methyl proton at C-29. Most of the other peaks corresponded to those of stigmast-4-en-3,6- dione and other similar compounds of this class reported from different sources (Akihisa et al., 1992; Tsuda and Schroepter, 1979). The multiplicity of carbon atoms in the ^{13}C-NMR ($CDCL_3$, 75 MHz) spectrum confirmed the suggested structure. The methyl carbons appeared at δc 12.24 (C-18), and δc 19.65 (C-19), 19.04 (C-21), 19.30 (C-27, and 12.12 (C-29). The upfield resonances of C-18 and C-19 at δc 12.24 and 12.12 suggested the location of the methoxy ester group at C-26 (Peres and Nagem, 1997; Chung et al., 2006; Li et al. (1999). The most deshielded carbonyl carbons at C-3, C-6 was assigned to the peak at δc 231 and 223, respectively. It shows the presence of Ene-di-One system in the molecule. The downfield shift of these carbonyl molecules compared to reference compound is possible only when the C-2 is attached with hydroxyl group. The peak at δc 72.1 for C-2 was assigned to Carbinolic carbon. The deshielded carbonyl carbon signal at δc 183.4 was for – $COOCH_3$ at C-26 and the peak at δc 64.87 for OCH_3 group. The deshielded signals at δc 161.7 and 122.0 were assigned to vinylic carbons at C-5 and C-4 of the Ring A, respectively (Akihisa et al., 1992; Tsuda and Schroepter, 1979; Panico et al., 1994). The molecular ion peak of S1 in FAB-MASS was found at m/z 509(M^+Na), suggesting the molecular formula as $C_{30}H_{46}O_5$ (Cal. 486.6972). The fragmentation pattern is characteristic of a steroid as it showed peaks at m/z 468(M-H_2O, 429(M+2H-$COOCH_3$), 395[M+H-Me-H_2O-COOH), 471(M-CH_3), 427(M-$COOCH_3$). 287(M-side chain), thus, confirming the number of carbon atoms in the side chain and the $COOCH_3$ group at C-26 carbon. Peaks at m/z 345,288 and 274 were formed due to (M-side chain), [M-(side chain+ cleavage of C_{16} and C_{17} bond)] and [M-(side chain+ ring D cleavage)], respectively (Chung et al., 2006; Li et al., 1999; McLafferty, 1973).

Thus, the IR, ^{1}H-NMR, ^{13}C-NMR and Mass-Spectral analysis along with physical properties established the identity of Compound S1 as 2β- hydroxy-Stigmasta- 4-en-3, 6-dione- methoxy ester (Scheme 1).

Conclusion

The results of the present investigation constitute the occurrence of 2β- hydroxy- Stigmasta- 4-en-3,6-dione methoxy ester type compound in plant kingdom. The title compound has been isolated from this plant for the first time.

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REFERENCES

Akihisa T, Hayashi Y, Petterson GW, Shimizu N, Tamura T (1992). 4-alpha methylvernosterol and other Sterols from Vernonia anthelmintica seeds. Phytochemistry 31(5):1759-1763.

Ali M (2001). Techniques in Terpenoid Identification. Birla Publications Delhi, India.

Bellamy LJ (1975). The Infrared Spectra of Complex Molecules. Chapman and Hall London.

Bheemasankara rao ch, Suseela K, Subba rao PV, Gopala KP, Subba raju GV (1984). Chemical examination of some Indian Medicinal Plants. Ind. J. Chem. 23B:787-788.

Chakabati B, Chaudhuri A, Choudhury PR (1968). Analysis of the essential oils of Artabotrys odoratissimus fruits and leaves. J. Indian Med. Assoc. 51(5):227-229.

Chakarabarti B, Chaudhuri A, Chowdhury PR J (1968). Antifertility effect of green leaves of *Artabotrys odoratissimus*. Indian Med. Assoc. 51:227.

Chopra RN, Nayar SL, Chopra IC (1956). Glossary of Indian Medicinal plants, antifertility effect of green leaves of *Artabotrys odoratissimu.* (C.S.I.R. Publication, New Delhi) 25.

Chung Ill-M, Ali M, Khan TD, Choung MG, Park HJ, Ahmed A (2006). Novel anthracene derivatives isolated from Rice Hulls of *Oryza sativa*. Bull. Korean Chem. Soc. 27(1):93-98.

Connoly JD, Haquc ME, Hasan CM, Haider SS (1994). Constituents of the stem bark of *Artabotrys odoratissimus* . Fitoterapia, 65(1):92-93.

Dewick P (1997). Medicinal Natural Products - A Bio-synthetic Approach. John Wiley and Sons Ltd. Chichester, England.

Fujita T, Liu D, Veda S, Takeda Y (1992). Xanthones from *Polygala tenuifolia*. Phytochemistry. Phytochemistry 31:3997-4000.

Haider SS, Hasan CM, Hussain CF (1991). Chemical constituents of stem bark of *Artabotrys odoratissimus*. J. Bangla Acad. Sci. 15(1):59-62.

Li W, Chan C, Leung H, Yeung H, Xiao P (1999). Xanthones from *Polygela caudata*. Phytochemistry. 51:953-958.

McLafferty FW (1973). Interpretation of Mass Spectra. Benjamin Inc. Massachusets. pp. 118-119.

Panico R, Powell WH, Richer JC (1994). IUPAC Commission on the Nomenclature of Organic Chemistry: A guide to IUPAC Nomenclature of Organic Compounds. Blackwell Scientific Publications Ltd., Oxford UK. Supersedes in part ref. 8.pp. 71-73.

Peres V, Nagem TJ (1997). Trioxygenated naturally occurring Xanthones. Phytochem. 44:191-214.

Perold GW, Rosenber MEK, Howard AS, Huddle PA (1978). Anthraquinones from *Artabotrys odoratissimus* leaves. J. Chem. Soc. Perkin Trans. 1:239.

SAS (1997). Institute SAS/ STAT user's Guide, SAS Institute, Cary, NC, 6.12.

Sharma M, Desiraju S, Chaurey D, Mehta BK (2002). GC-MS study of *Artabotrys odoratissimus* fatty oil (leaves). Grasas Aceites. 53(2):187-189.

Silverstein RM, Bassler GC, Morill TC (1984). Spectrometric Identification of Organic Compounds. John Wiley and Sons, New York 4th Edn. 181.

Singh JP, Singh AK, Ranjan R, (2009). Chemical constituents of *Artabotrys odoratissimus* (seeds). Rasayan J. Chem. 2(1):156-158.

Song HK, Ahn JK, Ahmad A, Hahn SJ, Kim SJ, Chung IM (2004). Identification of allelochemicals in rice root exudates at various phonological phases and their influence on barnyard grass. Allelopathy J. 13(2): 173-188.

Trivedi CP, Saxena SP, Emmanuel J (1971). Preliminary phytochemical and pharmacological studies on *Artabotrys odoratissimus*. Indian J. Med. Res. 59:635.