Isolation and Characterization of a Novel Compound from Antibiotic and Antioxidant Fraction from Extract of Stem Bark of Ventilago Maderaspatana (Garten).

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Abstract- *Ventilago maderaspatana* is a medicinal herb belonging to family Rhamnaceae. This herb is used in traditional medicine for Kapha, Dyspepsia, Colic disorder, Leprosy, Skin diseases and general disability. In our previous work we have noticed that methanolic extract of stem bark of *Ventilago maderaspatana* have exhibited strong antioxidant and significant antibiotic activity on gram negative bacteria and *Candida albicans* and the major phytochemicals phytosterols, flavonoids and alkaloids were noticed in bioactive extractive. During our present investigation on isolation of active constituents from bioactive fraction using chromatographic methods, we have isolated a pure compound designated as (E)-6-(3,4-dihydroxy-2-methyl-4-(2,6,6-trimethylcyclohex-2-enyl)but-1-enyl)-7-methoxy-2H-chromen-2-one, a novel coumarin derivative from analysis of UV, IR, proton NMR and Mass spectral data .We report this compound as a new natural product .

Index Terms- *Ventilago maderaspatana*, Phytochemical, UV, FTIR, ^{1}HNMR, ^{13}CNMR

I. INTRODUCTION

The stem bark of *Ventilago maderaspatana* has been used for thousands of years for its medicinal properties such as appetite stimulant, treatment for gastro intestinal infection, to lower blood glucose in diabetes, for treatment of certain type of cancer and viral infections[7]. It is not understood yet which active ingredients are responsible for clinical usefulness .With this background ,we have examined the extracts of stem bark for the presence of various phytochemicals and found that it is rich in phytosterols , while other metabolites such as alkaloids ,flavonoids and phenolic compounds were also detected in moderate quantities .We have also noticed significant broad spectrum antimicrobial activity of methanolic extract of stem bark against several gram-positive and gram-negative bacteria including pathogenic fungus *Candida albicans* . In in-vitro antioxidant assay it showed 100% anti scavenging activity in our earlier studies[10]. Therefore the methanolic extract of stem bark of *Ventilago maderaspatana* with promising antimicrobial and strong antioxidant property is undertaken for further investigation to isolate and characterize active ingredients for therapeutic use during the present studies. It resulted into isolation of a new coumarin derivative .Its method of isolation and various spectral data for structure elucidation is presented in this paper.

II. METHODS

Plant material

The stem bark of *Ventilago maderaspatana* was collected from forest of Chandrapur district, Maharashtra state, India and authenticated by PGTD, Dept of Botany RTMNU, Nagpur. It was washed, dried, powdered and stored for further studies.

Extraction procedure

Air dried and coarsely powdered stem bark material was extracted with solvent methanol using Soxhlet extraction apparatus by percolation for 8 hours under reflux. 100grams bark material yielded 8 gm of extract.

Solvent fractionation

1gm crude methanol extract was suspended in water: methanol (8:2) and partitioned successively with hexane and ethyl acetate solvents which upon concentration gave 0.104gm of hexane soluble fraction and 0.328gm ethyl acetate soluble fraction.

III. RESULTS AND DISCUSSION

All the chemicals used were of AnalaR grade. The solvents were dried and distilled before use acScording to standard procedures. For column chromatography silica gel mesh 60-120 Merck grade was used. TLC was performed on silica gel glass plates containing 60 GF-254 and visualization was achieved by UV light and iodine chamber. HPLC profile of the compound was obtained by using Instrument Shimadzu, UVspectrophotometer. UV-VIS analysis was carried on Jasco V550 spectrophotometer: FTIR spectrum was recorded in KBr medium on a Perkin–Elmer 783 spectrophotometer. Proton NMR spectrum was recorded in $CDCl_3$ and run at 300MHz on a Brucker Avance 300 spectrometer and chemical shifts are reported in parts per million (**δ**) downfield from Tetramehylsilane as internal standard. ^{13}C NMR spectrum was recorded in $CDCl_3$ and run at 75MHz on a Brucker Avance 300 spectrometer. ESI mass spectra was recorded on Micromass, Quattro LC using ESI software with capillary voltage 3.98 KV and ESI mode positive ion trap detector.

Column chromatography Isolation

After TLC analysis, the residue from ethyl acetate fraction (0.328g) was purified over a column of silica gel mesh 60-120, Merck grade using 4% ethyl acetate in n hexane. Homogeneous fractions were combined based on TLC and divided into two major fractionsA1 and A2. Fraction A1 was further purified by silica gel column using solvent mixture 4% ethyl acetate in hexane.It yielded two compounds which were recrystalized with hexane to get two pure compounds, Compound (1 0.023g) and compound 2 (0.004g). Compound 1 was obtained as a red brown semisolid with a Rf value 2.4 by TLC monitor and m.p. was 120-122^{0}C. Compound 1 was characterized using various spectral methods of analysis to arrive at the structure 1 assignable to it.

Table I: Analytical data of ethyl acetate residue of *Ventilago maderaspatana* using column chromatography

Elute from column Fraction no.	Mobile phase (ml)	TLC
1--5	hexane	Mixed spot
6--20	2% EA in hexane	Mixed spot
21-40	4% EA in hexane	Pure compound 1 (0.023g)
41-60	6% EA in hexane	Mixed spot
61-80	8% EA in hexane	Pure compound 2 (0.004g)
81-120	10% EA in hexane	Mixed spots

HPLC and UV analysis:

Compound 1 has shown λ_{max} at 292 nm in its UV-Vis analysis besides absorption in the visible region at λmax 434.5 nm with sholders was indicative of flavonoid or chromone compound with possible substitution or linkage with carotene like fragment.

Table II . Analytical data of analysis Ethyl acetate soluble residue of *Ventilago maderaspatana* using U.V. analysis

Sr. No.	λ_{max} (MeOH)	Absorbance
1	292	1.27416
2	434.5	0.52208
3	661	0.04423

FTIR spectrum of compound 1

In order to study the structure of compound 1, we have recorded the FTIR spectrum. It shows at high wave numbers the absorption band at 3311cm^{-1} assignable to CHOH (OH) stretch, 2920 cm^{-1} assignable to CH stretch of CHOH, 2880 cm^{-1} is attributed to OCH_3 , 1705 cm^{-1} is assignable to α-βunsaturated ketone. He absorption band at 1448 cm^{-1} is attributed to gemdimethyl CH rock.The absorption band 767-828 cm^{-1} is assignable to methyl CH rocks.

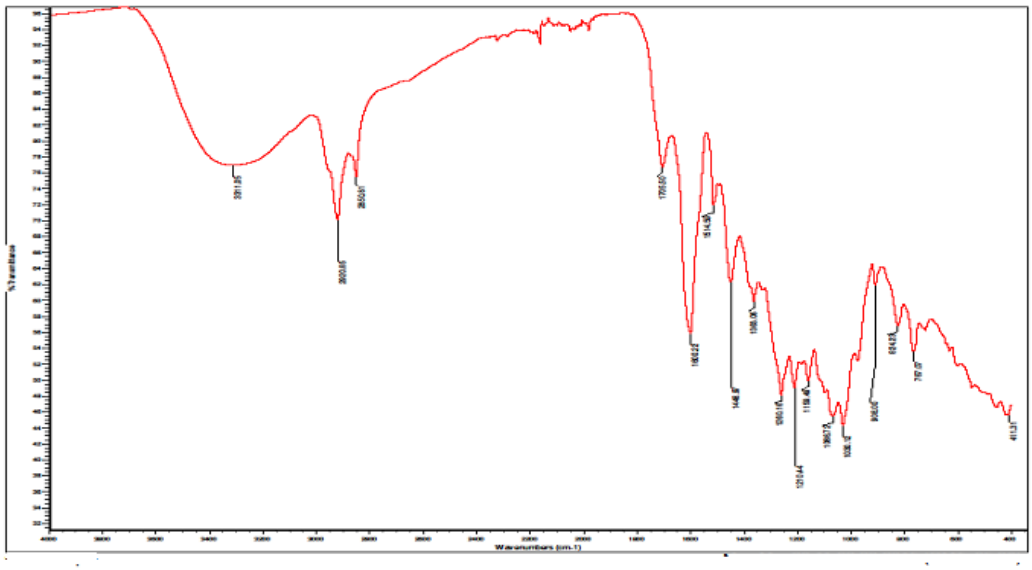


Figure 1: FTIR spectrum of compound 1

Mass spectral analysis

Its ESIMS mass spectrum shows fragment ions at m/e 382 and 368for the fragments M^+-OH+H and M^+ –OCH_3+H respectively accounting for molecular ion M^+ at m/e 398 corresponding to molecular formula C_{24} H_{30} O_5 for compound 1. Fragment ion peaks noticed at m\e 382 and m\e 368 for the fragments M^+-OH +H and M^+-OCH_3+H respectively accounting for the molecular ion M^+ at m/e 398 noticed in ESIMS mass spectrum. M^+ m/e 398 corresponds to molecular formula $C_{24}H_{30}O_5$ for compound -1

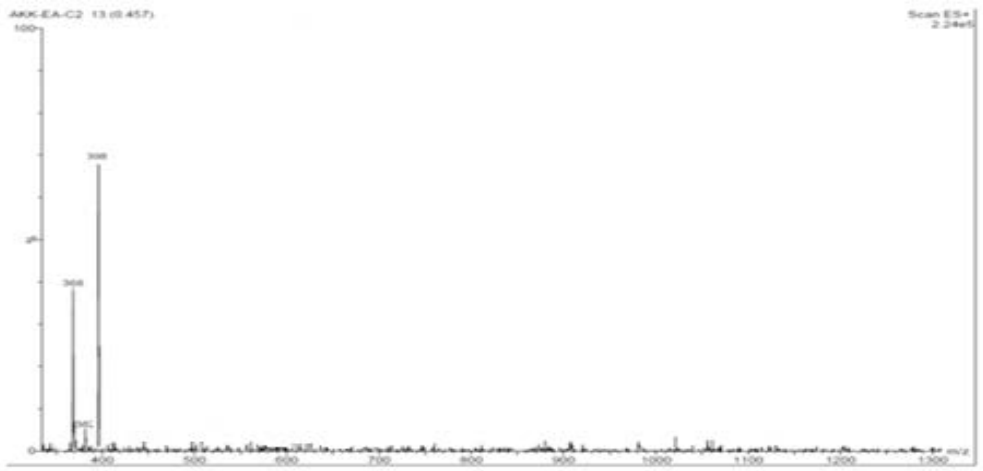


Figure 2: ESIMS mass spectrum of compound 1

^{1}H NMR spectrum of compound 1

Its proton nmr displayed singnals at δ 6.58,7.08,7.53 and 7.76 assignable for aromatic protons H-3, H-4 H-5 H-8 respectively besides signal at δ 3.49 assignable to OCH_3 protons probably at C-7 very commonly noticed in coumarin compounds and absence of aromatic proton signal at C-6 is suggestive of substitution at C-6. Other proton chemical shifts pertaining to C-6 side chain include presence of 2 olefinic protons assignable at δ 7.34 and 5.39 , adjacent CHOH coupled protons appeared at δ 4.33 and 4.02 , 2 olefinic methyls at δ 2.04 and 1.61 and gem-dimethyl at δ 0.99 and 0.88 indicative of a geranyl butyl side chain accounting for C_{14} H_{23} O_2 at C-6 of coumarin with $C_{10}H_7O_3$ in agreement with molecular formula $C_{24}H_{30}O_5$

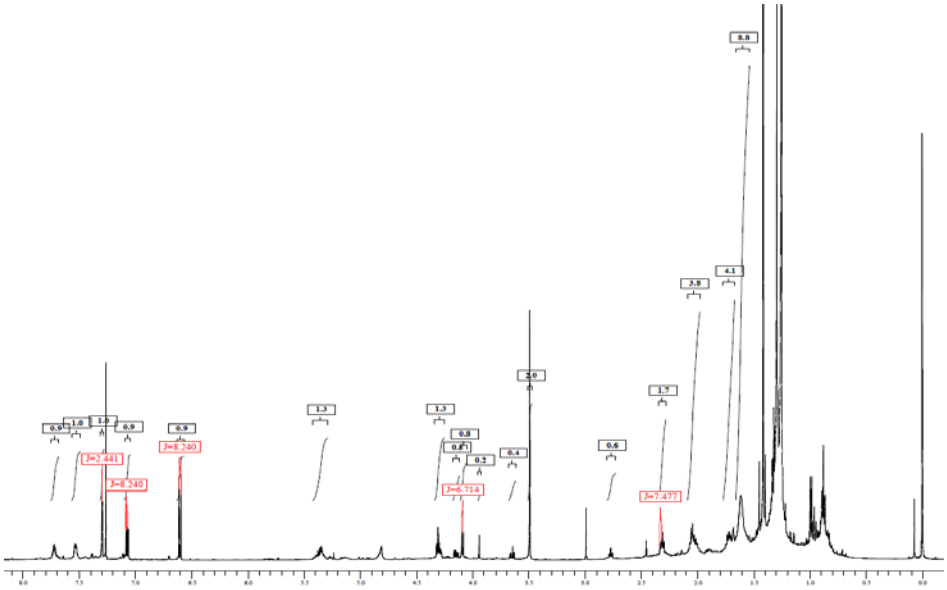


Figure 3. ^{1}H NMR spectrum of compound1

Table III. Analytical data of ^{1}H NMR spectrum of compound 1

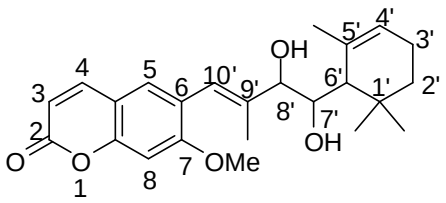
Proton	Chemical shift (δ ppm)	Coupling constant(*J*= Hz)
H-3	6.58	d, 8.24
H-4	7.08	d, 7.08
H-5	7.53	m
H-8	7.76	m
H-4'	5.39	m
H-6'	2.33	dd, 7.47, 10.05
H-8'	4.02	d 6.71
H-7'	4.33	dt 6.71, 10.22
H-10'	7.34	s, br
O-CH_3 -7	3.49	s
CH_3-9'	2.04	s
CH_3-5'	1.61	s, br
gem CH_3 -1'	0.88	m
gem CH_3 – 1'	0.99	m

^{13}C NMR spectrum of compound 1

^{13}C NMR spectrum of compound 1 was recorded by employing TMS as internal reference standard and CDCl3 as solvent at ambient temperature.

Out of 24 Carbon signals, nine signals are corresponding to basic coumarin skeleton (δ 111.5, 111.9, 111.4, 120.8, 124.1, 144.1, 153.1, 161.2and 161.6) The spectrum also showed two olefinic carbon signals at δ 22.5.Signal at δ 65 corresponds to methoxy C, two signals at δ 71 ,74 assignable to secondary alcoholic C. Two signals at δ 20, 21 correspond to geminal dimethyl carbon.Two signals at δ 19.3 and 20.5 are assignable to two gemdimethyl groups.Signal at δ 25.4 corresponds to olifenic methyl group. Two signals at δ 120.86, 119.10 were assigned olefinic carbon at position 10' and position 4'. Signals at δ 29.40 and δ 30.20 correspond to CH_2 carbon at position 2' and at position 3' .

By comparing the literature[3], ESIMS mass spectrum, FTIR spectrum of compound 1,the proton and carbon data of compound 1, we propose that compound 1 has moleculer formula $C_{24}H_{30}O_5$ supported by ESIMS mass spectral analysis. Structure of compound 1 is designated as (E)-6-(3,4-dihydroxy-2-methyl-4-(2,6,6-trimethylcyclohex-2-enyl)but-1-enyl)-7-methoxy-2H-chromen-2-one.



(1)

IV. CONCLUSION

In this paper we report isolation and characterization of a novel coumarin derivative from the stembark of *Ventilago maderaspatna* with substitution at C6 reported for the first time .

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REFERENCES

[1] Bush, T., E. & Scott , G. W. 1981 Fluorescence of distyrylbenzenes. J. Phys. Chem. 85, 144–146.

[2] Brain, K.R., Terner, T.D. 1975.The practical evaluation of Phytopharmaceuticals. Wright Scientechnica, Bristol, 4-35.

[3] Edoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4(7): 685-688.

[4] Ernst, L. (1976) 13C NMR spectroscopy of polycyclic aromatics. VI. Coumarin and methylcoumarins. J. Magn. Reson. 21, 241–246.

[5] Harborne JB. Phytochemical methods – A guide to modern techniques of plant analysis. Edn 3, Chapman & Hall publications, 1998.

[6] Hsieh, T. J., Su, C. C., Chen, C. Y., Liou, C. H. & Lu, L. H. 2005 Using experimental studies and theoretical calculations to analyze the molecular mechanism of coumarin, p-hydroxybenzoic acid, and cinnamic acid. J. Mol. Struct. 741, 193–199.

[7] http://3n Wikipedia. Org/wiki/ventilago/uses

[8] Johnson, L.R.F, Jankowski, W.C. 1972 Carbon 13 NMR spectra. Wiley-Interscience, New York

[9] Kashman, Y., Gustafson ,K. R., Fuller, R. W., Cardellina ,J. H., McMahon ,J.B., M. J., Currens , Buckheit ,R. W., Hughes S. H., Cragg ,G.M. & Boyd M. R. 1992.. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, Calophyllum lanigerum. J. Med. Chem. 35, 2735–2743.

[10] Kawade, A.B,Batra, R.J.,Weginwar,R. G., Akkewar, D.M.,Gond, G.S.,Aparna ,Y. 2014 International Journal of Res. In Pharmacy and Chemistry, 4(1) 78-82.

[11] Kumar, S., Giri, R., Mishra, S. C. & Machwe, M. K. 1995. Photophysical characteristics of the laser-dye 7-dimethylamino cyclopenta C coumarin. Spectrochim. Acta A Mol. Biomol. Spectrosc. 51, 1459–1467.

[12] Kus, N , Breda, S, Reva I., Tasal E., Ogretir C.and Fausto R 2007. FTIR Spectroscopic and Theoretical Study of the Photochemistry of Matrix-isolated Coumarin .Photochemistry and Photobiology, 83: 1237–1253

[13] Khandelwal, K.R. Practical Pharmacognosy techniques and experiments, Edn 8, Nirali Prakashan publications, 2001.

[14] Mohammad, A. , Bhawani,S. &Sharma ,A.S. 2010.Analysis of Herbal Products by Thin-layer Chromatography: A Review International Journal of Pharma and Bio Sciences V1(2).

[15] Munshi, P. & Row T. N. G. 2005. Exploring the lower limit in hydrogen bonds: Analysis of weak C-H ... O and C-H ... p interactions in substituted coumarins from charge density analysis. J. Phys. Chem. A 109, 659–672.

[16] Nyquist, R. A. and Settineri, S. E. 1990. Infrared study of coumarin in different solvent systems. Appl. Spectrosc. 44, 791–796.

[17] Okwu, D.E. 2001. Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet. J., 14: 160-162. Antherden, L.M. 1969. Textbook Of Pharmaceutical Chemistry, 8th edn., Oxford University Press, London, pp. 813-814.

[18] Ratnakar, P., Murthy, P.S. 1995. Purification and mechanisms of action of antitubercular principle from garlic (Allium sativum) active against isoniazid susceptible and resistant Mycobacterium tuberculae H37RV. Indian Journal of Clinical Biochemistry 10, 14–18.

[19] Stray, F. 1998. The Natural Guide to Medicinal herbs And Plants. Tiger Books International, London, pp. 12-16.

[20] Smyth, W. F., Ramachandran ,V. N., Hack, C. J., Joyce, C.& O'Kane ,E. 2006 A study of the analytical behaviour of selected synthetic and naturally occurring coumarins using liquid chromatography, ion trap mass spectrometry, gas chromatography and polarography and the construction of an appropriate database for coumarin characterization. Anal. Chim. Acta 564, 201–210.

[21] Swami, K.D., Bisht, N.P.S., 1996. Constituents of Ficus religiosa and F. infectoria and their biological activity. Journal of the Indian Chemical Society 73 (11), 631.

[22] Tanaka, T., Moriita, A., Nanaka, G., 1991. Tannins and related compounds C III. Isolation and characterisation of new monomeric, dimeric and trimeric ellagitannins, calamanisanin and calamanins A, B, and C from Terminalia caamansani. Chemical Pharmacy Bulletin 38, 60.

[23] Vogel, A.I., 1958. A Textbook of Practical Organic Chemistry. Longman, London, pp. 90–92.

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