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INVESTIGATIONS ON SECONDARY METABOLITES FROM *CRATEVA RELIGIOSA* G. FORST.–A RARE MEDICINAL PLANT OF VIDARBHA REGION (M.S.) INDIA

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ABSTRACT

Crateva religiosa G. Forst. is commonly called as 'Viawarna' belongs to family Capparaceae which is used as folkloric medicine to cure various types of ailments. In the present study, the roots of this plant were extracted successively in three different solvents, Chlorofrom, Dichloromethane and 50% Ethanol by soxhletion method. Gas Chromatography – Mass Spectrometry (GC-MS) method was used to separate and identify the individual compounds in all the three extracts. The compounds were identified by comparing mass spectra of the separated compounds with those in the NIST library. Fifteen important secondary metabolites were identified in the roots of this plant which were having several biological activities thereby verifying the folkloric claims of this plant part.

KEY WORDS: Secondary metabolites, *Crateva religiosa* and GC-MS.

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INTRODUCTION

Crateva religiosa also known as *C. adansonii* DC commonly called as Sacred Garlic Pear or Vaiwarna belongs to family Capparaceae. It is commonly called as Varun[1] and its trade name is three leaved capper.[2] It is native to Japan, Australia, Southeast Asia and several south Pacific Islands[3-5] In India it is found in Pennisular India, Western India, Gangetic Plains, and Eastern India upto Tripura and Manipur. It is moderate sized deciduous tree and mostly found along the bank of rivers & streams and near to temple side[6-7] and it is a rare medicinal plant in Maharashtra.[8]This plant specimen has been collected during floristic surveys in various geographical areas in India and morphologically has been described.[9-12] It is an important medicinal plant and has been used in Indian Ayurvedic medicine for various ailments. The plant parts which are used for medicinal purposes are root bark, stem bark and leaves.[13-15] The plant has been claimed to be used as an antipyretic, a rubefacient and to counter irritation as well as to manage conditions such as bladder and kidney diseases, skin diseases, snake and insect bites.[16-22] The leaves are applied externally to relieve pain in joints; the fresh juice of leaves is used for the relief of ear ache, eye infection and anodyne in toothache. Powder of bark is used in itch, epilepsy, and asthma.[23] In order to verify these folkloric claims, the present study was carried out.

MATERIALS AND METHODS

Collection and Identificaton

The plants were collected from Amravati district of Vidarbha region, Maharashtra which were morphologically identified and authenticated by taxonomist Professor Dr. S.P. Rothe. The voucher specimens were deposited in the herbarium of Department of Botany, Vidyabharati Mahavidyalaya, Amravati, (M.S.) India. During the collection, roots which were infected etiolated or having any diseased condition was removed accordingly and fresh plant material i.e., roots were shade dried and then grinding into fine powder for further studies.

Extraction

The extraction was done by using Soxhlet method[24] in three different solvents viz. Chloroform , 50% Ethanol, and Dichloromethane successively. After 24 hours, the extracts were filtered and concentrated up to 5ml using rotatory vacuum evaporator at room temperature. Then these crude samples were used for GC - MS analysis.

GC-MS analysis

GC-MS analysis was carried out by using gas chromatography-high resolution mass spectrometer. 2 µl of prepared extracts was employed for this analysis. GC-MS analysis was carried using Alegent Hp 7880 with column of 30 meter length, with 0.25 mm internal diameter and 0.32 thickness. Helium gas was used as carrier gas at constant flow rate of 1ml/minute. Injector temperature was set at 50 ^{0}C. the Oven temperature were programmed from 50 ^{0}C to 280 ^{0}C at 10 ^{0}C /minute to 200^{0}C then 10 ^{0}C/ 3 minutes to 250 ^{0}C ending with a 5 minutes isothermal at 280 ^{0}C. The sample was injected in split mode as 10:80.

Identification of compounds

Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of unknown compounds was compared with the spectral data of known compounds present in spectral libraries (NIST).The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The chromatograms of *C. religiosa* roots were obtained by GC-MS analysis after the successive Soxhlet extractions in Chloroform, Dichloromethane and 50% Ethanol. All the major peaks were matched with standard compounds and fragments in NIST database for the identification of probable compounds present in these samples. Figure 1, 2 and 3 are the chromatograms of Chloroform extract, Dichloromethane extract and 50% Ethanol extract respectively. Seven phytochemical compounds were identified in Chloroform extract, Nineteen compounds in Dichloromethane extract and 15 compounds in 50% Ethanol extract respectively as shown in (Table 1, 2, and 3) and also retention time (RT), compound name, peak area (% of compound in extract), molecular weight (MW) and molecular formula (MF) for every compound is depicted. Table 4 shows the list of secondary metabolites found in all the three extracts of *C. religiosa* root with compound name, its nature and biological activity. It was found that Chloroform extract was rich in Steroids like Campestrol, Stigmasterol, Sitosterol and Phenol (methyl salicylate), in Dichloromethane extract many terpenes like (+)-Camphor, Menthol, β-Caryophyllene, α-Caryophyllene, Ar-Curcumene, β –Sesquiphellandrene, Ar-tumerone, Curlone, and a cardiac glycoside (Strophanthin) etc were present, while as in 50% Ethanolic extract a steroid Drebyssogenin- F , Sugars D-Melezitoze & L-Glucose and some fattyacids, esters, hydrocarbons were identified.

Table 1
Compounds identified in Chloroform extract

S. No.	RT	Name of Compound	% of Peak Area	MW	MF
1	6.76	Methyl salicylate	29.24	152	$C_8H_8O_3$
2	12.93	1-Hexadecene	2.04	224	$C_{16}H_{32}$
3	18.45	n-Hexadecanoic acid	3.75	256	$C_{16}H_{32}O_2$
4	18.96	1-Nonadecene	1.72	266	$C_{19}H_{38}$
5	28.80	Campesterol	10.84	400	$C_{28}H_{48}O$
6	29.91	Stigmasterol	18.48	412	$C_{29}H_{48}O$
7	32.0	Y-Sitosterol	19.70	414	$C_{29}H_{50}O$

Table 2
Compounds identified in Dichloromethane extract

S. No.	RT	Name of Compound	% of Peak Area	MW	MF
1	7.11	4.4-Ethylenedioxy-1-pentylamine	1.14	145	$C_7H_{15}NO_2$
2	10.76	(+)-Camphor	1.78	152	$C_{10}H_{16}O$
3	11.61	1-Menthol	0.21	156	$C_{10}H_{20}O$
4	15.86	Eugenol	0.36	164	$C_{10}H_{12}O_2$
5	17.15	β-Caryophyllene	0.86	204	$C_{15}H_{24}$
6	17.88	α-Caryophyllene	1.72	204	$C_{15}H_{24}$
7	18.44	Ar-Curcumene	1.41	202	$C_{15}H_{22}$
8	19.25	β –Sesquiphellandrene	1.06	204	$C_{15}H_{24}$
9	20.7	1,3,3,4- Trimethyl -4-(methylphenyl) Cyclopentanol	1.34	218	$C_{15}H_{22}O$
10	21.07	12-Oxabicyclo (9,1,0) dodeca-3,7-diene, 1,5,5,8-tetramethyl, [1R-R*,3E,7E,11R*)]-	1.035	220	$C_{15}H_{24}O$
11	22.08	Ar-tumerone	3.24	216	$C_{15}H_{20}O$
12	22.77	Curlone	2.76	218	$C_{15}H_{22}O$
13	25.23	β -k-Strophanthin	3.79	710	$C_{36}H_{54}O_{14}$
14	27.39	1,2-Benzenedicarboylic acid, butyl 8-methylnonyl ester	18.67	362	$C_{22}H_{34}O_4$
15	31.3	9,12-Octadecadienoic acid (Z,Z)	10.35	280	$C_{18}H_{32}O_2$
16	31.45	Trans-13-Octadecenoic acid	17.06	282	$C_{18}H_{34}O_2$
17	36.34	Heptacosane	5.98	380	$C_{27}H_{56}$
18	40.52	10,12,14,-Nonacosatriynoic acid	3.36	426	$C_{29}H_{46}O_2$
19	42.56	Heptacosane	23.13	380	$C_{27}H_{56}$

Table 3
Compounds identified in 50% Ethanol extract

S. No.	RT	Name of Compound	% of Peak Area	MW	MF
1	4.08	5-Hexen-3-ol,2,2,4-trimethyl	12.82	142	$C_9H_{18}O$
2	6.42	Acifloctin	0.01	146	$C_6H_{10}O_4$
3	7.73	D-Melezitose	0.01	504	$C_{18}H_{32}O_{16}$
4	14.79	3-Heptanone, 4-methyl	15.56	128	$C_8H_{16}O$
5	16.34	Bicyclo[3.1.0]hexane-2-undecanoic acid, methyl ester	8.72	280	$C_{18}H_{32}O_2$
6	16.62	D-Melezitose	5.34	504	$C_{18}H_{32}O_{16}$
7	16.87	L-Glucose	1.63	180	$C_6H_{12}O_6$
8	18.19	Methyl 8-oxooctanoate	12.28	172	$C_9H_{16}O_3$
9	18.33	1-Methyl-1-[prop-2-enyl]-1-silacyclopentane	0.01	156	$C_{18}H_{16}OSi$
10	18.50	n-Hexadecanoic acid	10.26	256	$C_{16}H_{32}O_2$
11	21.81	Trans-13-Octadecenoic acid	21.16	282	$C_{18}H_{34}O_2$
12	22.03	Mandenol	0.02	308	$C_{20}H_{36}O_2$
13	22.12	Oleic acid	3.36	282	$C_{18}H_{34}O_2$
14	27.0	9,12,15-Octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl) oxy) methyl] ethyl ester, (Z.Z.Z)-	0.01	496	$C_{27}H_{52}O_4Si_2$
15	36.66	Drebyssogenin f	6.77	506	$C_{28}H_{48}O_8$

Table 4
List Secondary metabolites found in Crateva religiosa roots

S. No.	Name of Compound	Category	Biological activity
1	Methyl salicylate	Phenol	Anti-inflammatory and pain killing
2	Campesterol	Steroid	Antioxidant; Hypocholesterolemic[25]
3	Stigmasterol	Steroid	Inhibits tumor promotion[26], anti-HIV reverse transcriptase, Anti-inflammatory[27], inhibit Na^+ K^+ pump ATPases and influence prostrate metabolism and growth[28]
4	ϒ-Sitosterol	Steroid	Anticancerous[29], Uterotonic, Estrogenic[30], Antioxidant, Anti-inflammatory and Antitumor activities[31]
5	Drebyssogenin f	Steroid	Not evaluated for any particular function
6	(+)-Camphor	Cyclic Monoterpene	Inhibitors of micro-organisms, reduce the risk of fungal infection[32]
7	1-Menthol	Cyclic Monoterpene	Flavours and fragrances in food.
8	Eugenol	Phenylpropanoid	Carminative, Antiseptic[33]
9	β-Caryophyllene	Sesquiterpene	Antinociceptive[34]
10	α-Caryophyllene	Sesquiterpene	Fragrances and cosmetics
11	Ar-Curcumene	Sesquiterpene	Insect repellant[35]
12	β –Sesquiphellandrene	Sesquiterpene	Flavoring agent
13	Ar-tumerone	Sesquiterpene	Antidode for snake bite[36] Anti-apoptotic[37]
14	Curlone	Sesquiterpene	Antioxidant, Antibacterial[38]
15	β -k-Strophanthin	Cardiac Glycoside (aglycone)	Cardiotonic, Toxic , Heart arresting[39]

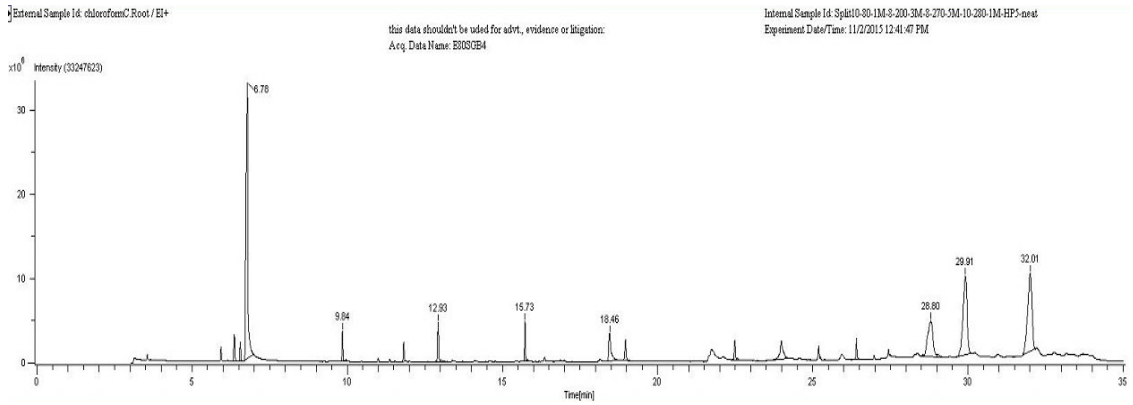

Figure 1
Chromatogram of Chloroform extract

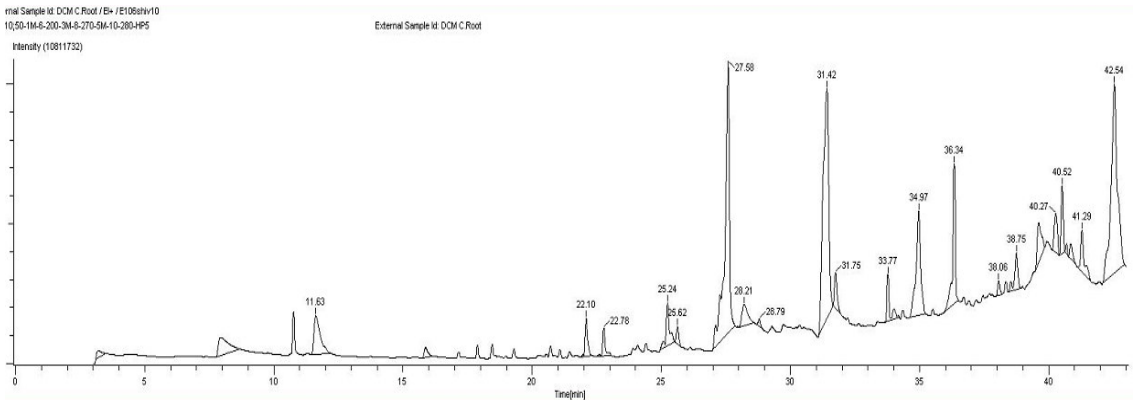

Figure 2
Chromatogram of Dichloromethane extract

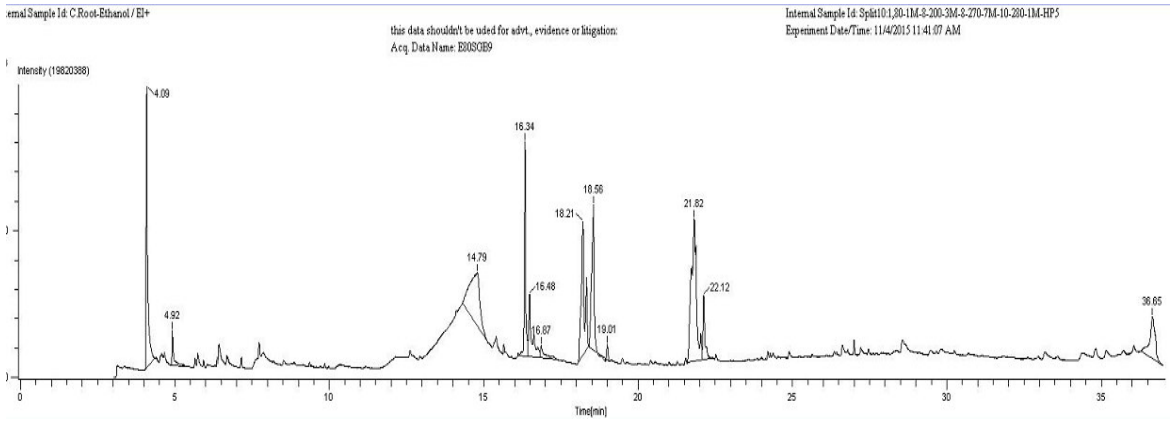

Figure 3
Chromatogram of 50% Ethanol extract

CONCLUSION

The results of this study suggest that *Crateva religiosa* G. Forst. should be considered as a great source of phytoconstituents which can be useful in natural alternative therapy for patients with various disorders either alone or in combination with other suitable agents. The identified secondary metabolites compounds have the potential for curing various diseases and can be used in various pharmaceutical and herbal medicines.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Williamson M. Major herbs of Ayurveda. Churchill Livingston publication: Elsevier Science Ltd; 2002; p. 111-6.
2. Pullaiah T. Encyclopedia of world medicinal plants. 1st ed. Regency Publication; 2006. Vol. 1, p. 256.
3. Jacobs M. The Genus Crateva (Capparaceae). Blumea, 1964;12:196-7.
4. Sharma BD. Flora of India (F. India). Calcutta: Botanical Survey of India. 1993.
5. Achigan-Dako E, Pasquini M, Assogba Komlan F, Sognigbe N, Dansi A & Ambroseoji B. Traditional vegetables in Benin: Diversity, distribution, ecology, agronomy and utilization. Bangor: IFS, 2009. P. 252.
6. Panda H. Handbook of Medicinal Plants. Delhi :Asia Pacific Publication; 2004. p. 112.
7. Warrier PK. Indian medicinal Plants: A compendium of 500 species. Arya V.S. (Ed.), France: Orien Publication; 1995. Vol. 3, p: 280.
8. Survase SA. and Raut SR. Ethnobotanical study of some tree medicinal plants in Marathwada, Maharashtra. Journal of Ecobiotechnology. 2011;3(2):17-21.
9. Cooke T. The Flora of the Presidency of Bombay., vol I & II. Culcatta : Botanical Survey of India; 1967.
10. Singh NP, Lakshminarasimhan P, Karthikeyan S. and Prasanna PV. Flora of Maharashtra State, Dicotyledones. Vol. II. Calcutta: BSI; 2001.
11. Naik VN. & Associates. Flora of Marathwada. Vol. 1&2, Aurangabad: Amrut Prakashan; 1998.
12. Patil DA. Flora of Dhule and Nandurbar Districts, Dehradun: Bishen Sing Mahendra Pal Sing; 2003.
13. Bhatachargee SK. Hand book of medicinal plants, Jaipur: Aaviscar Publication and Distributors; 2001. p. 117.
14. Anonymous. Crataeva: Wealth of India. New Delhi : CSIR Publication; 1987. Vol. 2, p. 366.
15. Nadkarni KM. India Materia Medica. 3rd ed. (Revised and enlarged by Nadkarni, AK.) Bombay: Popular Prakasham; 1976. Vol. 1 and 2.
16. Quisumbing E. Medicinal plants of the Philippines. Department of Agriculture & Natural Resources Technical Bulletin 16, Manilla, Philippine Islands: Manila Bureau of Printing; 1951.
17. Irvine FR. Woody Plants of Ghana. With Special Reference to Their Uses. London: Oxford University Press; 1961.
18. Gill LS. Ethnomedical Uses of Plants in Nigeria. Benin, Nigeria: University of Benin Press; 1992.
19. Ashton MS, Gunatilleke S, De Zoysa N, Gunatilleke N, Dasanayake MD and Wijesundra, S. A field guide to the common trees and shrubs of Sri Lanka. Sri Lanka: The Wildlife Heritage Trust; 1997.
20. Gupta P, Patel N, Bhatt L, Zambare GN, Bodhankar SL, Jain BB. et al. Antiurolithiac effect of petroleum ether extract stem bark of *Crataeva adansonii* in rats. Pharmaceutical Biology. (2006;44(3):160-65.
21. Maruthupandian M. and Mohan VR. Observations of ethnomedicinal plants from Sirumalai Hills in Western Ghats of Tamilnadu, India. Journal of Herbal Medicine and Toxicology. 2010;4(2):89-92.
22. Ogbole OO, Gbolade AA. and Ajaiyeoba EO. Ethnobotanical survey of plants used in treatment of inflammatory diseases in Ogun State, Nigeria. European Journal of Scientific Research, 2010;43(2):183-191.
23. Sivarajan VV, and Balachandran I. Ayurvedic Drugs and their Plant Sources. Delhi : Oxford and IBH Publishing Company Pvt. Ltd; 1994.
24. Nikhal SB, Dambe PA, Ghongade DB. and Goupale DC. Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. International Journal of Pharmaceutical Sciences. 2010; 2(1):30-32.
25. Sureshkumar P. Phytochemical assessment on various extracts of *Calotropis gigantean* (L.) R. Br. Through GC-MS. Int J. Pharm Bio Sci. 2013;4(2):803 – 10.
26. Wagay NA. and Rothe SP. Investigations on secondary metabolites of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Leaves using GC-MS. Journal of Pharmacognosy and Phytochemistry. 2016; 5(5):114-18.
27. De Oliveira GG., Pereira junior JADeS, Bastos IVGA, Sampaio Filh RCO, Lopes NP. and De melo SJ. Phytochemical Investigation of Chloroform extract from Root, Stem and Leaf of *Adenocalymma imperatoris-maximilianii* (Wawra) L.G. Lohmam (Bignoniaceae). Int J Pharm Bio Sci., 2014;5 (3): 70 – 8.
28. Bombardelli, E. and Morazzoni, P. *Prunus africana* (Hook.f.) Kalkm. Fitoterapia. 1997;68, pp: 205-18.
29. Scholtysek C, Krukiewicz AA, Alonso JL, Sharma KP, Sharma PC. and Goldmann W H. Characterizing components of the Saw Palmetto Berry Extract (SPBE) on prostate cancer cell growth and traction. Biochem Biophys Res. Commun. 2009;379(3):795-8.
30. Duke JA, Godwin MJB, DuCdlier J, Duke PK. Handbook of Medicinal Herbs. 2nd edition, USA : CRC press; 2002.
31. 31. Duke J. Dr. Duke's ethnobotanical and phyto-chemistry database; 1998. Available from: www.ars- grin.gov/duke/
32. Saxena M, Saxena J, Nema R, Singh D. and Gupta A. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6):168-82.
33. Visht Sharad and Chaturvedi Swati. Isolation of Natural Products. CPR. 2012;2(3):584-99.
34. Alalan L, Al- Shammaa I. and Al-Nouri AS. Isolation and identification of six terpenes extracted from leaves of *Inula viscosa* (L.) grown in Syria. Journal of Chemical and Pharmaceutical Research, 2016;8(1):27-31.
35. Bleeker PM, Diergaarde PJ, Ament K., Guerre J, Weidner M, Schutz S, et al. The role of specific tomato volatiles in tomato-whitefly interaction. Plant Physiology. 2009;151:925-35.

36. Ferreira LA, Henriques OB, Andreoni AA, Vital GR, Campos MM, Habermehl GG. and de Moraes VL. Antivenom and biological effects of ar-turmerone isolated from *Curcuma longa* (Zingiberaceae). Toxicon. 1992; 30(10):1211-8.
37. Aratanechemuge Y, Komiya T, Moteki H, Katsuzaki H, Imai K. and Hibasami H. Selective induction of apoptosis by ar-turmerone isolated from turmeric (*Curcuma longa* L) in two human leukemia cell lines, but not in human stomach cancer cell line. Int J Mol Med. 2002; 9(5):481-4.
38. Nir Y, Potasman I, Stermer E, Tabak M, and Neeman I. Controlled trial of the effect of cinnamon extract on *Helicobacter pyroli*. J Pub Med. 2000;5:94-7.
39. Naudé TW. The occurrence and significance of South African cardiac glycosides. Journal of the South African Biological Society. 1977;18:7.