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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] |

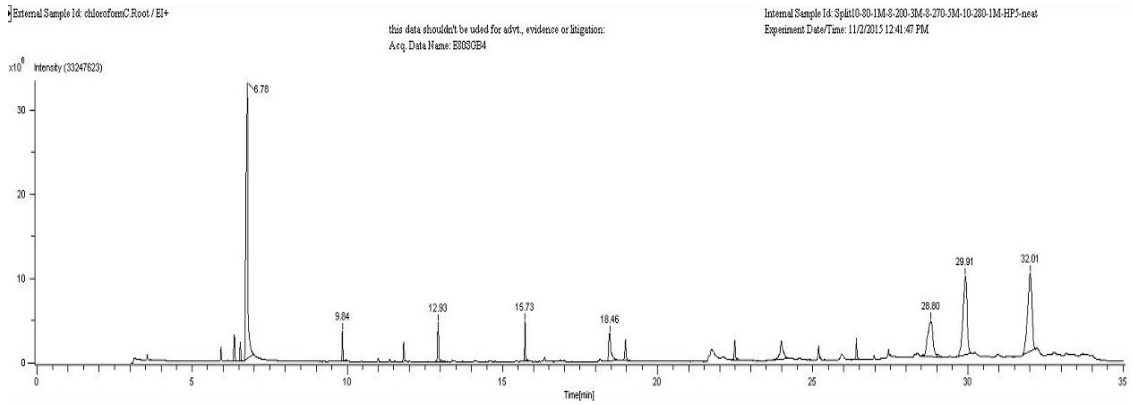


**Figure 1**
***Chromatogram of Chloroform extract***

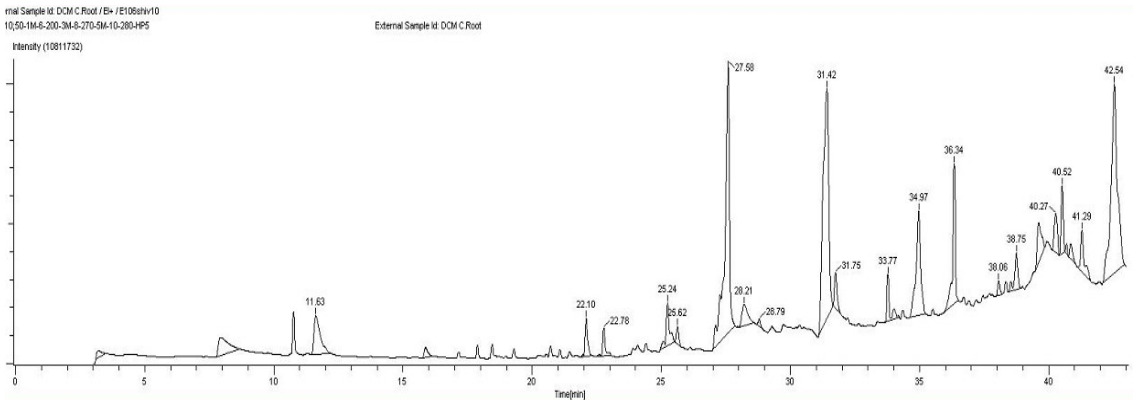


**Figure 2**
***Chromatogram of Dichloromethane extract***

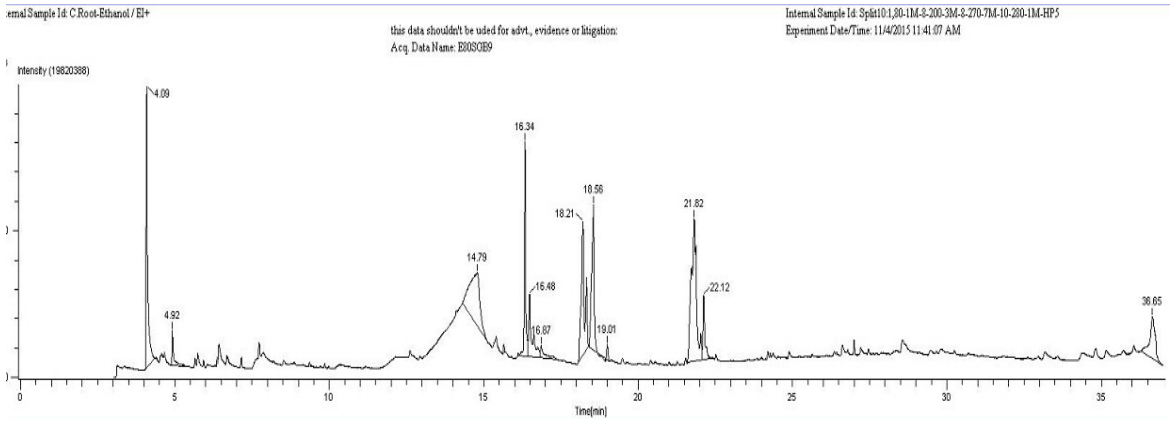


**Figure 3**
***Chromatogram of 50% Ethanol extract***

## 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.

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