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# Profiling of secondary metabolites and antimicrobial activity of *Crateva religiosa* G. Forst. Bark - A rare medicinal plant of Maharashtra India

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**Key words:** GC-MS, Secondary metabolite profiling, Anti-microbial activity.

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## Abstract

In the present study Profiling of Secondary metabolites and Antimicrobial activity of Stem Bark of *Crateva religiosa* was carried out for validating the ethno medicinal claims. Stem Bark of this plant was analyzed for Physiochemical study, Organoleptic study and Fluorescent analysis. Qualitative and Quantitative analysis of major secondary metabolites was also carried out using standard procedures. Stem bark was extracted successively using Chloroform, Dichloromethane and 50% Ethanol as solvents which were analyzed by Gas Chromatography – Mass Spectrometry (GC-MS) method to separate and identify the individual compounds in extracts. Antimicrobial activities of all three extracts of understudy part was tested against 4 pathogenic bacterial strains and two fungal strains. The results of antimicrobial activity were compared with the results of standard antibiotics. The physiochemical results determined that percentage of moisture content 6.70 ± 0.59, Ash content 22.18 ± 1.17, Highest Extractive values 15.85 ± 0.21 were found in 50% Ethanol extracts. The qualitative analysis showed presence of various secondary metabolites among which major groups were quantified. By GC-MS analysis the different phyto compounds were identified among which 9 were secondary metabolites and were placed as profile of secondary metabolite in Stem bark. All three solvent extracts showed significant activity against bacterial strains while as chloroform extracts were inactive against fungal strains at 10mg/ml concentration. The results suggest that this plant has vast variety of photochemical which can be used as effective remedy for various ailments and drug formulations in future. The ethnic claims of this plant were also verified by the present study.

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## Introduction

*Crateva religiosa* G. Forst. also known as *Crateva adansonii* DC. is called as Sacred Garlic Pear or Temple plant and belongs to the family Capparaceae. It is commonly called as Varun (Williamson, 2002). And its trade name is three leaved capper (Pullaiah, 2006). It is native to Japan, Australia, Southeast Asia and several south Pacific Islands (Jacobs, 1964; Sharma, 1993). In India it is found in Pennisular India, Western India, Gangetic Plains, and Eastern India upto Tripura and Manipur. It is a rare medicinal plant in Maharashtra (Survase and Raut, 2011). 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 (Bhatachargee, 2001; Anonymous, 1987; Nadkarni, 1976). Powder of bark is used in itch, epilepsy, and asthma (Sivarajan and Balachandran, 1994). The bark is useful in disorders of urinary organs, urinary tract infections, pain and burning micturition, renal and vesical calculi (Nwosu, 2000). The importance of this medicinal plant can also be imagined as, a postal stamp was issued by the Indian postal Department to commemorate this tree.

The plant has been studied for suppressing various immune factors (Bani *et al.,* 2006); Antimycotic potential (Sahoo *et al.,* 2008); Ant mutagenic activity (Chichioco-Hernandez *et al.,* 2009); Wound healing (Ajali *et al.,* 2010); Anti-inflammatory activity (Abdullahi *et al.,* 2012); Antimicrobial activity (Agboke *et al.,* 2011); Antioxidant activity (Pandey *et al.,* 2013). The profiling of Secondary metabolites and the antimicrobial activity on understudy microorganisms was checked for the first time. The Present study was also undertaken to validate these ethnomedicinal uses and to know about the chemicals present in the Stem bark of this plant especially Secondary metabolites which are of most medicinal importance.

## Materials and methods

*Collection of plant material*

The plant material was collected by planning the frequent visits to the wild habitat where the plants were growing abundantly rather than agricultural or local habitat. The plant material was collected from Amravati Taluka, Amravati. These plants were collected during all periods of their growth periods from January to October, 2014.

*Identification of Plants*

The collected plants were morphologically identified with the help of standard floras (Cooke, 1967; Kamble and Pradhan, 1988; Dhore, 1989; Naik, 1998; Singh and Karthikeyan, 2001; Almeida, 2001) and authenticated by taxonomist Professor Dr. S.P. Rothe. The voucher specimens were deposited in the herbarium of Department of Botany, Vidyabharati Mahavidyalya, Camp, Amravati, Maharashtra, India. Later on the plant material was subjected to further experimentation.

*Ethno medicinal Importance*

During collection, the ethno-medicinal data of above selected plants was also obtained from tribal healers, local Vaidoos, Old aged Villagers and medicinal practitioners (Jain and Sakalmi, 1999).

*Preparation of Powder*

The cleaned plant material was subjected to shade drying for about one week. After one week the plant material was transferred to oven at 45$^{o}$C for four hours. This helped to remove the moisture content from plant material after shade drying.

This dried plant material was then converted to powder form using electric mixture grinder. Finally, prepared powder was stored in air tight plastic bags and was used for further experimentation.

*Analytical methods*

The procedures recommended in Indian Pharmacopoeia (Anonymous, 1966) and Gupta *et al.*, 2013 were followed for analytical or physiochemical study.

*Organoleptic Evaluation*

The Organoleptic evaluation of understudy plant part was done with the parameters mentioned in Indian Pharmacopoeia (Anonymous, 1966).

*Fluorescent Chemical Analysis*

The Fluorescent behavior analysis for understudy plant part was carried out by using standard methods of Pratt and Chase, 1949.

**Phytochemical Analysis**

*Qualitative Phytochemical Analysis*

*Preparation of Extracts*

Decoction (for water only) and Soxhlet extraction methods were used for extracting phytoconstituents (Tiwari *et al.*, 2011; Nikhal *et al.*, 2010).

*Preliminary/Qualitative analysis*

The qualitative analysis for secondary metabolites of understudy plant part was done in solvents; Water, Dichloromethane, 50% Ethanol and Chloroform using standard methods (Kokate, 2005; Harborne, 1998; Sadashivan and Manickam, 2005; Wallis, 1990). The extracts were analyzed for eight secondary metabolite groups namely Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids, Tannins, and Terpenoids. The extracts were analyzed for the presence of secondary metabolites by the following tests. The procedure of these tests is given in Table 1.

*Quantitative Photochemical Analysis*

Extracts in which preliminary qualitative analysis showed presence of specific groups of secondary metabolites like Falconoid, Alkaloids, Saponins and Phenolics were subjected to quantitative analysis. The crude quantification of major phytochemicals present in understudy plants was done using precipitation and spectrophotometric method as per suitability. Each sample was analyzed in triplicates. The standard procedures were followed for Alkaloids (Harborne, 1973), Flavonoids (Bohm and Kocipai- Abyazan, 1994), Saponins (Obdoni & Ochuko, 2001) and Phenolics (Vermerris *et al.*, 2006).

*Gas Chromatography – Mass Spectrometric analysis*

*Preparation of Extracts*

The extracts for Gas chromatography – Mass spectroscopy analysis were prepared using three solvents viz. Chloroform, Dichloromethane and 50% Ethanol by Soxhlet method. After extraction in Soxhlet apparatus for 24 hours the extracts were filtered and concentrated to 5ml using rotatory vacuum evaporator at room temperature, and then stored at -20˚C temperature (Wagay *et al.,* 2016).

The analysis was carried out using gas chromatography- high resolution mass spectrophotometer. Samples were worked out at SAIF, IIT Powai, Mumbai, Maharashtra, India.

**Table 1.** Preliminary Tests for detection of Secondary metabolites.

| S. No | Secondary metabolites | Name of Test | Methodology and Confirmation of compounds |
|---|---|---|---|
| 1. | Alkaloids | Wagners test | Add 2ml filtrate with 1% HCl. Then add 1ml of the solution with 6 drops of Wagner's reagent. Brownish-red precipitate indicates presence of alkaloids. |
| | | Mayers test | To the 2-3ml of filtrate, few drops of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids. |
| 2. | Flavonoids | Sodium hydroxide test | Treat the extract with dilute NaOH, followed by addition of dilute HCl. A yellow solution with NaOH, turns colorless with dilute HCl indicating presence of Flavonoids. |
| | | Lead acetate test | To 2-3ml of extract, lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids. |
| 3. | Glycosides | Killer-killiani test | To the 5ml of extract, 1ml of conc. $H_2SO_4$, 2ml of Glacial acetic acid and 1 drop of $FeCl_3$ solution was added. Appearance of Brown ring shows the presence of cardiac glycosides. |
| | | Fehlings test | Extract was hydrolyzed by HCl and neutralized with NaOH followed by addition of Fehling's solution 'a' and 'b' in 1:1 proportion, produces a red precipitate, indicating presence of glycosides. |
| 4. | Phenols | Phenols test | Spot the extract on a filter paper. Add a drop of phoshomolybdic acid reagent and expose to ammonia vapors. Blue coloration of the spot shows presence of phenols. |
| 5. | Saponins | Frothing/ Foam test | Add 0.5ml of filtrate with 5ml of distilled water and shake well. Persistence of frothing shows presence of Saponins. |
| 6. | Steroids | Salkowaski test | To 2ml of extract, 2ml of chloroform and 2ml of conc. $H_2SO_4$ was added. By Shaking this solution chloroform layer turned red and acid layer showed greenish yellow fluorescence indicating presence of Steroids. |
| | | Liebermann | To 1ml of extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of |

| S. No | Secondary metabolites | Name of Test | Methodology and Confirmation of compounds |
|---|---|---|---|
| | | burchard's test | concentrated sulphuric acid. Dark green coloration shows presence of steroids. |
| 7. | Tannins | $FeCl_3$ test | Add few drops of 5% $FeCl_3$ solution to the extract, appearing of deep blue black color indicates presence of Tannins. |
| 8. | Terpenoids | Salkowaski test | To 2 ml of extract, 2ml of chloroform and 2ml of conc. $H_2SO_4$ was added. On shaking chloroform layer turned red and acid layer shows greenish yellow fluorescence indicating presence of terpenoids. |

**Table 2.** GC-MS facility centre and Features of Machine used for Analysis.

| Analyzed at | SAIF, IIT Powai, Mumbai |
|---|---|
| Machine used | AlegentHp 7880 |
| Sample used | 2 µl |
| Column length/ Internal diameter/ Thickness | 30 meters / 0.25 mm / 0.32mm |
| Carrier gas | Helium Gas |
| Injector temperature | 100 °C |
| Flow rate | 1µ l/ minute |
| Oven temperature | 50-280°C |
| Heating rate | 10°C / min |
| Split mode | 10:80 |

*Identification of compounds*

All the three samples which were analyzed by gas chromatography – mass spectroscopy were identified using National Institute of Standard and Technology (NIST) Database. The chromatogram, retention times, fragmentation pattern, m/z values, base peak, mass peak, peak intensities etc. were obtained through GC-MS analysis. The identification is primarily based on two parameters, retention times and fragmentation pattern of compounds. Along with these m/z values, matching number of peaks are used to confirm the compound identification. The information acquired through this was name, structure, molecular weight, molecular formula and relative quantity of compounds. The online database which aided in the identification of compounds are METLIN – The metabolite mass spectral database, Mass Bank, Respect for photochemical and Golmmetabolome database.

*Antimicrobial Activity*

Extracts of understudy plant part were tested for antimicrobial activity against four pathogenic bacterial strains and two fungal strains. All the experiments were performed in triplicates.

*Preparation of Extracts for Antimicrobial activity*

The dried powder of understudy plant part (10g) were extracted with Chloroform, 50% Ethanol and Dichloromethane by keeping the flask on rotary-shaker at 150 rpm for 24 hrs. After 24 hours, the supernatant was filtered through Whatman filter paper No. 41. The solvent suspension was completely evaporated using vacuum while, the residue obtained was dissolved in 1% $DMSO_4$ (Dimethyl Sulphoxide) and the concentration was made as 10mg/ml from every plant part extract, which wasused for further studies. The antibacterial activity was carried out using well diffusion method described by Perz *et al.*, 1990; Satish *et al.*, 2008; and Nitha *et al.*, 2012 and disc diffusion method described by Bauer *et al.*, 1966; Alzoreky *et al.*, 2003. The bacterial strains were obtained from Department of Microbiology, Shri Shivaji Science College, Akola, Maharashtra.

The antifungal activity was carried out using disc diffusion methods as described by Bauer *et al.*, 1966; Alzoreky *et al.*, 2003. Fungal Cultures were obtained from Department of Biotechnology, SGB Amravati University, Amravati, Maharashtra. The antimicrobial activity for tested organisms was assessed by measuring the diameter of the growth - inhibition zone in millimeters (including well diameter and paper disc diameter) of plant extracts. The results of extracts were compared with the results of standard antibiotics.

**Results and disscussion**

*Crateva religiosa* G. Forst. belongs to Family: Capparaceae (Caper family) and Commonly called as Varuna or Viawarna or Nirvala.

*Morphological Characters*

*Crateva religiosa* G. Forst. Issmall to moderate sized tree with much- branched head. Bark grey, smooth horizontally wrinkled; wood is yellowish white,

turning light-brown when old. Leaves deciduous, pinnatelytrifoliolate, clustered at the ends of branchless with a common petiole 4-8 cm long at the summit of which are tree leaflets; leaflets 5-16 × 4-6cm, ovate lanceolate or obovate, acute or acuminate, attenuated at the base, entire, glabrous on both surfaces, pale beneath and reticulately veined, the lateral leaflets oblique at the base with a rather slender point at the tip; petiolules 6-10mm long. Flowers many, in dense terminal corymbs, greenish-white, pedicels 2.5-4.5cm long, Stout, and glabrous. Sepals petaloid, small, distant, ovate, and acute.

Petals ovate or oblong (including the claw) nearly 2.5 by 1cm, claw up to 6mm long, very narrow. Stamens purplish longer than the petals spreading. Gynophore nearly 5cm long, terete, smooth. Ovary ellipsoid; stigma flat. Fruit globose, 3-5 centimeters in diameter, woody, smooth or scurfy berry, with hard and rough rind, on the thickened gynophores. Seeds about 10 centimeters in length, numerous, kidney-shaped, embedded in yellow pulp, nearly smooth & brown. Base cuneate or sub acute, margins entire, apex gradually caudate. Flowers: March –May.

*Ethnomedicinal Properties*

Lodhas (local Tribe in Vidarbha region) prescribe stem bark paste with paste of 3-9 peppers (3:1) as laxative. The decoction of bark is useful in fevers, to relieve vomiting and symptoms of gastric irritation, the decoction is prepared by bruising and boiling 4 ounces of it in 1 ½ pints of water till reduced to 1 pint and then strained and cooled; the dose is from 2 to 4 ounces. It is also used in snake bite. Juice of bark is given to women after childbirth.

Powdered bark is useful in urinary and renal tubules, gastro-intestinal and uterine affections. Fresh juice of stem bark with seed powder of black peppers (3:1) is given to women as contraceptive. Other ethnic communities use fresh bark paste to stimulate appetite. Externally the bark and leaves pounded and tied in a cloth are applied as fomentation.

**Analytical/ Physio-chemical study results**

**Table 3.** Analytical values.

| S. No | Parameter studied | | %age value (w/w) |
|---|---|---|---|
| | | | Stem |
| 1. | Moisture content | | 6.70 ± 0.59 |
| 2. | Total Ash | | 22.18 ± 1.17 |
| 3. | Acid soluble ash | | 24.77 ± 0.36 |
| 4. | Acid insoluble ash | | 74.97 ± 0.27 |
| 5. | Water soluble ash | | 20.43 ± 0.57 |
| 6. | Water insoluble Ash | | 80.2 ± 0.52 |
| 7. | Extractive values in | Chloroform | 5.33 ± 0.10 |
| | | 50% Ethanol | 15.85 ± 0.21 |
| | | Dichloromethane | 3.24 ± 0.13 |

Note: Percentage mean (n=3) ± SD.

**Powder study results**

**Table 4.** Organoleptic evaluation.

| S. No. | Particulars | Observation |
|---|---|---|
| 1. | Color of powder | Light yellowish grey |
| 2. | Odor | Odorless |
| 3. | Taste | Tasteless |
| 4. | Texture | Rough |

**Table 5.** Fluorescent behavior of *Crateva religiosa* G. Forst. Stem powder on treatment with different chemical reagents.

| S. No. | Powder + Reagent used | Stem | |
|---|---|---|---|
| | | Visible Light | UV Light |
| 1. | Powder as such | Light yellowish brown | Yellow |
| 2. | Powder + Conc. $H_2SO_4$ | Black | Black |
| 3. | Powder + Conc. $HNO_3$ | Orange red | Red |
| 4. | Powder + Conc. HCl | Brown | Dark green |
| 5. | Powder + 10% NaOH | Light green | Light green |
| 6. | Powder + 1 N HCl | Cream white | White cream |
| 7. | Powder + Iodine solution | Dark brown | Dark brown |
| 8. | Powder + 5% $FeCl_3$ | Yellow Fluorescent | Fluorescent greenish yellow |
| 9. | Powder + KI | Transparent | Transparent |
| 10. | Powder + 1 N $HNO_3$ | Cream white | Cream |
| 11. | Powder +1 N $H_2SO_4$ | Cream white | Cream |
| 12. | Powder + Ethyl acetate | Transparent water | Transparent water |

**Phytochemical Results**

**Table 6.** Qualitative phytochemical analysis.

| S. No | Constituent | Chemical tests | STEM | | | |
|---|---|---|---|---|---|---|
| | | | W | D | E | C |
| 1 | Alkaloids | Wagners test | +++ | +++ | +++ | +++ |
| | | Mayers test | +-+ | +-+ | +-+ | +++ |
| 2 | Flavonoids | Sodium hydroxide test | +++ | +++ | +++ | --- |
| | | Lead acetate test | --- | --- | +-- | --- |
| 3 | Glycosides | Killer killiani test | --- | --- | --- | --- |
| | | Fehlings test | +++ | --- | +++ | --- |
| 4 | Phenols | Phenols test | --- | +++ | --- | +++ |
| 5 | Saponins | Froathing / Foam test | +++ | --- | --- | --- |
| 6 | Steroids | Salkowaski test | --- | +++ | --- | +++ |
| | | LB test | --- | +++ | --- | +++ |
| 7 | Tannins | $FeCl_3$test | --- | --- | --- | --- |
| 8 | Terpenoids | Salkowaski test | --- | +++ | +++ | +++ |

Note '+'= Present and '–'= Absent

Where, W= Water; D= Dichloromethane; E= 50% Ethanol; C= Chloroform respectively.

The qualitative photochemical screening of *Crateva religiosa* G. Forst. Stem Barkin four extracts i.e., Water, Dichloromethane, 50% Ethanol and Chloroform showed that there was prominently presence of phytoconstituents like Alkaloids, Flavonoids, Phenols, Glycosides, Saponins, Steroids and Terpenoids in all the three understudy plant parts. However, Tannins were absent in all the part extracts of *Crateva religiosa* G. Forst. Water and 50% Ethanol extracts showed presence of most phytoconstituents followed by Chloroform and Dichloromethane.

*Quantitative phytochemical analysis*

The crude content of major phytochemical compounds in *Crateva religiosa* G. Forst. Stem Bark were determined (Table 7).

It was found that among all the four tested phytochemicals, the plant showed higher level of Flavonoids then Alkaloids which are also closely followed by Saponins. The content of Phenols was in least percentage in the *Crateva religiosa* G. Forst stem Bark.

**Table 7.** Quantitative phytochemical analysis in Stem of *Crateva religiosa* G. Forst.

| S. No. | Phytochemicals | % of Crude Content (g / 100 gms of dry sample) |
|---|---|---|
| 1. | Flavonoids | 1.18 ± 0.24 |
| 2. | Alkaloids | 1.90 ± 0.17 |
| 3. | Saponins | 1.28 ± 0.25 |
| 4. | Phenols | 1.06 ± 0.17 |

Note: Percentage mean (n=3) ± SD.

### Chromatographic Study results

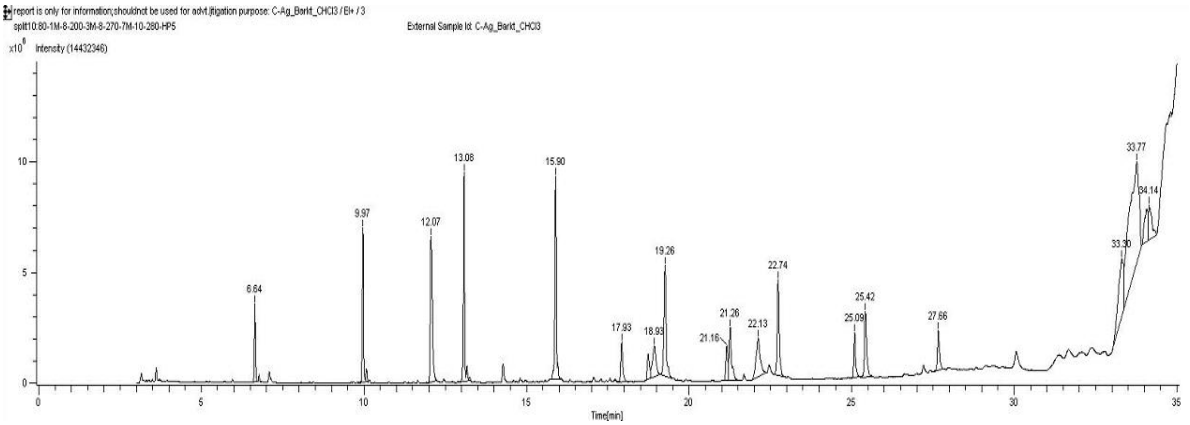



**Fig. 1.** Chromatogram of Chloroform extract of *Crateva religiosa* G. Forst. Stem.

**Table 8.** Compounds identified in the Chloroform extract of *Crateva religiosa* G. Forst. Stem.

| S. No. | RT | Name of Compound | Peak Area % | MW | MF |
|---|---|---|---|---|---|
| 1. | 6.64 | 1-Dodecene | 2.10 | 168 | $C_{12}H_{24}$ |
| 2. | 9.97 | 1-Tetradecene | 4.72 | 196 | $C_{14}H_{28}$ |
| 3. | 12.07 | Phenol,2,4-bis(1,1-dimethylethyl)- | 7.38 | 206 | $C_{14}H_{22}O$ |
| 4. | 13.08 | 1-Tetradecene | 6.72 | 196 | $C_{14}H_{28}$ |
| 5. | 15.90 | 1-Hexadecene | 7.32 | 224 | $C_{16}H_{32}$ |
| 6. | 17.93 | Hexadecanoic acid, methyl ester | 1.89 | 270 | $C_{17}H_{34}O_2$ |
| 7. | 19.26 | 3-Octadecene, (E)- | 6.11 | 252 | $C_{18}H_{36}$ |
| 8. | 21.26 | 9-Octadecanoic acid, methyl ester, (E)- | 3.15 | 296 | $C_{19}H_{36}O_2$ |
| 9. | 22.13 | Oleic acid | 3.85 | 282 | $C_{18}H_{34}O_2$ |
| 10. | 22.74 | 1-Nonadecene | 4.73 | 266 | $C_{19}H_{38}$ |
| 11. | 25.09 | S-Indacene, 1,2,3,5,6,7-hexahydro-1,1,5,5-tetramethyl-4,8-bis(3-methylbutyl) | 1.89 | 354 | $C_{26}H_{42}$ |
| 12. | 25.42 | 1-Docosene | 3.39 | 308 | $C_{22}H_{44}$ |
| 13. | 27.66 | 9-Hexacosane | 2.03 | 364 | $C_{26}H_{52}$ |
| 14. | 33.77 | Lup-20(29)-en-3-one | 25.80 | 424 | $C_{30}H_{48}O$ |

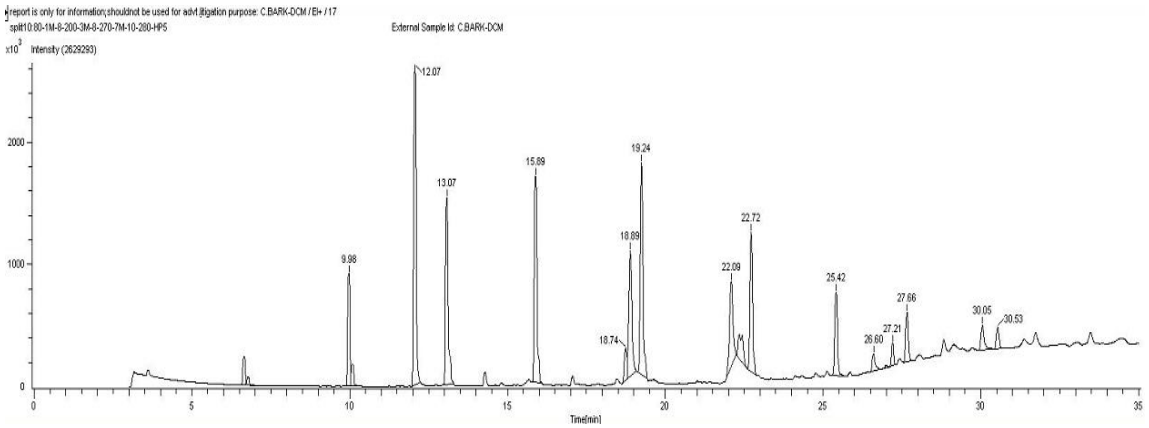



**Fig. 2.** Chromatogram of Dichloromethane extract of *Crateva religiosa* G. Forst. Stem.

**Table 9.** Compounds identified in the Dichloromethane extract of *Crateva religiosa* G. Forst. Stem.

| S. No. | RT | Name of Compound | Peak Area % | MW | MF |
|---|---|---|---|---|---|
| 1. | 6.66 | 1-Dodecene | 1.40 | 168 | $C_{12}H_{24}$ |
| 2. | 9.98 | 1-Tetradecene | 5.58 | 196 | $C_{14}H_{28}$ |
| 3. | 12.07 | Phenol,2,4-bis(1,1-dimethylethyl)- | 16.73 | 206 | $C_{14}H_{22}O$ |
| 4. | 13.07 | 1-Tetradecene | 10.95 | 196 | $C_{14}H_{28}$ |
| 5. | 15.89 | 1-Hexadecene | 11.75 | 224 | $C_{16}H_{32}$ |
| 6. | 18.89 | n- Hexadecanoic acid | 8.40 | 256 | $C_{16}H_{32}O_2$ |
| 7. | 22.09 | Trans-Octadecanoic acid | 6.15 | 282 | $C_{18}H_{34}O_2$ |
| 8. | 25.42 | 1-Docosene | 4.78 | 308 | $C_{22}H_{44}$ |
| 9. | 27.66 | 1-Docosene | 2.66 | 308 | $C_{22}H_{44}$ |

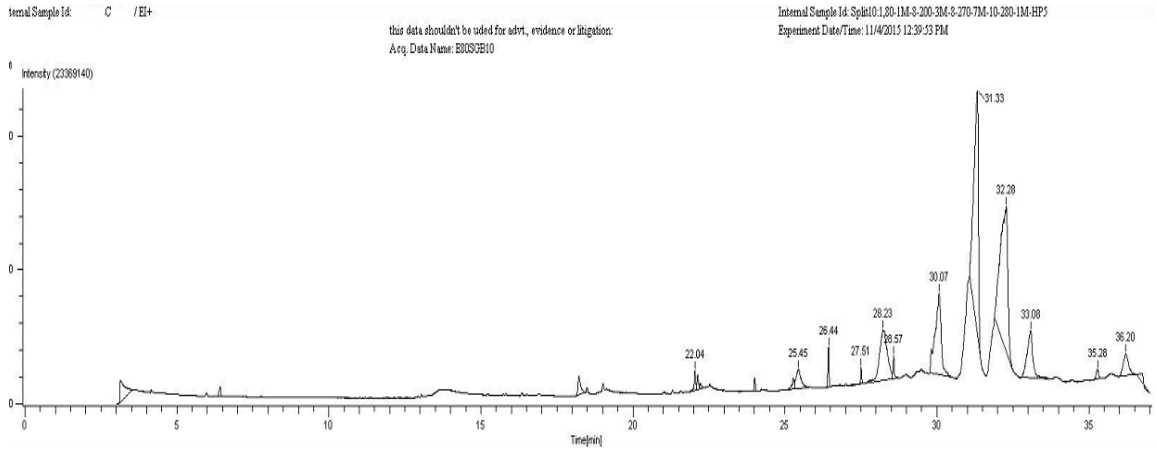



**Fig. 3.** Chromatogram of 50% Ethanol extract of *Crateva religiosa* G. Forst. Stem.

**Table 10.** Compounds identified in the 50% Ethanol extract of *Crateva religiosa* G. Forst. Stem.

| S. No. | RT | Name of Compound | Peak Area % | MW | MF |
|---|---|---|---|---|---|
| 1. | 6.42 | Menthol | 0.37 | 156 | $C_{10}H_{20}O$ |
| 2. | 18.23 | Strophanthin | 1.33 | 710 | $C_{36}H_{54}O_{14}$ |
| 3. | 22.04 | 9,12-Octadecanoic acid, ethyl ester | 0.74 | 308 | $C_{20}H_{36}O_2$ |
| 4. | 25.45 | Stigmasterol | 2.37 | 412 | $C_{29}H_{48}O$ |
| 5. | 28.23 | β-Sitosterol | 9.43 | 414 | $C_{29}H_{50}O$ |
| 6. | 30.07 | Lup-20(29)-en-3-one | 12.48 | 424 | $C_{30}H_{48}O$ |
| 7. | 31.33 | Lupeol | 27.70 | 426 | $C_{30}H_{50}O$ |
| 8. | 32.28 | Drebyssogenin f | 27.24 | 506 | $C_{28}H_{42}O_8$ |
| 9. | 33.08 | Pregn-5-en-one,11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-oxobutoxy)-(3β,11α,12β,14β)- | 6.68 | 506 | $C_{28}H_{42}O_8$ |
| 10. | 36.20 | Giganteumgenin N | 2.97 | 506 | $C_{30}H_{50}O_6$ |

**Table 11.** Secondary metabolite profile of *Crateva religiosa* G.Forst. Stem Bark.

| S. No | Name of Compound | Category |
|---|---|---|
| 1 | Stigmasterol | Steroid |
| 2 | 1-Menthol | Monoterpene/Terpenoid |
| 3 | β-k-Strophanthin | Cardiac Glycoside/Aglycone |
| 4 | Drebyssogenin f (or Pregn-5-en -one,11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-oxobutoxy)-(3β,11α,12β,14β)- | Glycoside / (Steroidal glycoside) |
| 5 | Phenol,2,4-bis(1,1-dimethylethyl)- | Phenol |
| 6 | Lup-20(29)-en-3-one (Lupenone) | Triterpene/Terpenoid |
| 7 | β-Sitosterol | Steroid |
| 8 | Lupeol | Triterpene/Terpenoid |
| 9 | Giganteumgenin N (Barrigenol R1) or (Olean-12-ene-3,15,16,21,28-hexol) | Saponi/Triterpenesapogenin |

**Table 12.** Antibacterial activity of *Crateva religiosa* G. Forst. Stem Bark showing zone of Inhibition in mm.

| Bacterial strain | Chloroform extract | 50% Ethanol extract | DCM extract | Antibiotics | | | |
|---|---|---|---|---|---|---|---|
| | | | | S | C | A | O |
| *Staphylococcus aureus* | 8 | * | * | 20 | * | 22 | 22 |
| *Sarcina maxima* | 10 | 20 | * | 32 | * | 24 | 38 |
| *Serratia marcescens* | 14 | 18 | 18 | 38 | * | 29 | 28 |
| *Pseudomonas aeruginosa* | 20 | 16 | 18 | 40 | 18 | 16 | 14 |

Where, * = 0 mm; 'S' = Streptomycin; 'C'= Ciprofloxacin; 'A'= Amoxycillin; and 'O' = Of loxacin.

**Table 13.** Antifungal activity of *Crateva religiosa* G. Forst. Stem Bark showing zone of Inhibition in mm.

| Fungal Organism | Chloroform extract | 50% Ethanol extract | DCM extract | Antibiotics | | | |
|---|---|---|---|---|---|---|---|
| | | | | H | I | C | F |
| *Aspergillus niger* | * | 8 | 18 | 18 | 14 | 17 | 16 |
| *Aspergillus flavus* | * | 10 | * | 20 | 40 | 36 | 30 |

Where, * = 0 mm; 'H' = Hexaconazole; 'I' = Itraconazole; 'C' = Clotrimazole and 'F' = Fluconazole.

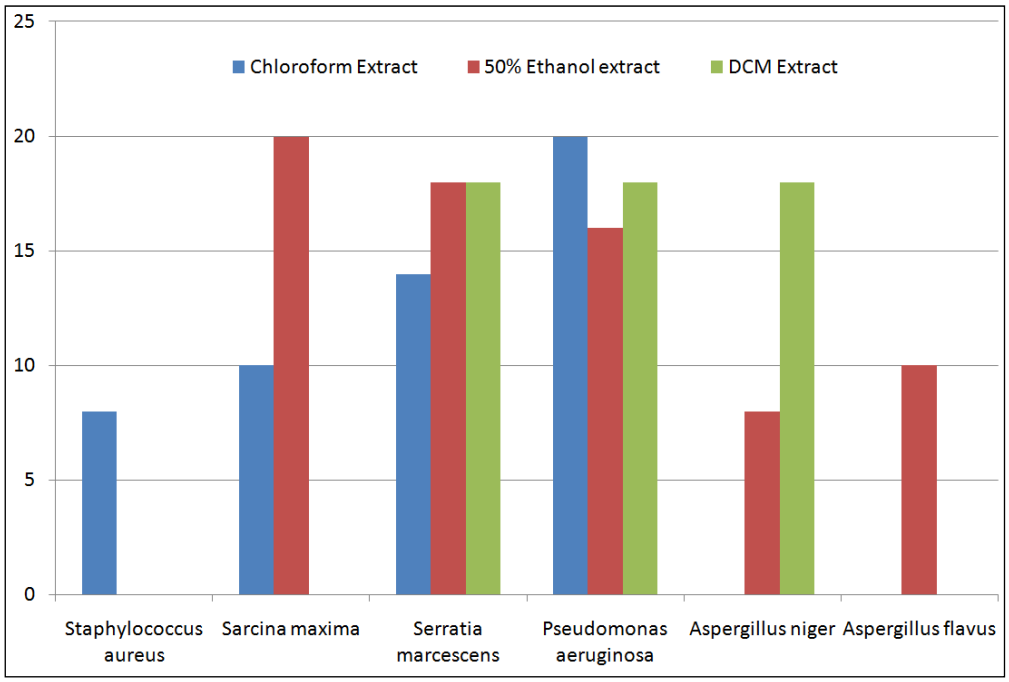
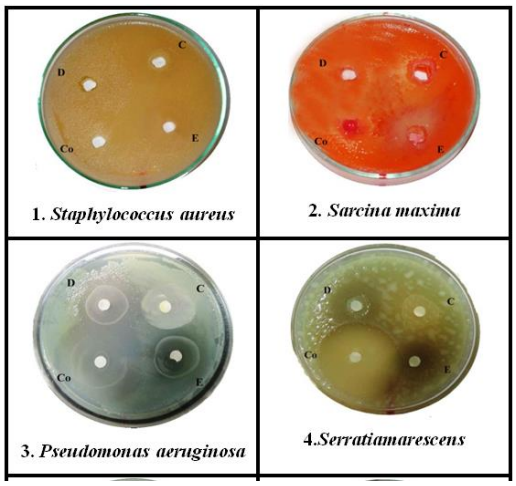



**Fig. 4.** Antibacterial and Antifungal activity of *Crateva religiosa* G. Forst. Stem Bark extracts showing zone of inhibition in mm (Graphical).



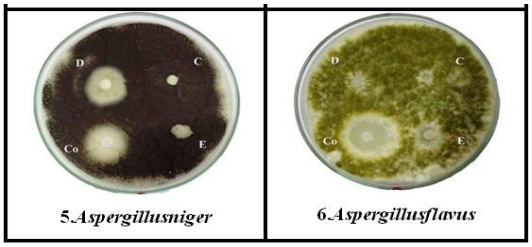




**Fig. 5.** The antibacterial and antifungal activity of *Crateva religiosa* G. Forst. Stem Bark extracts by using Disc and Well diffusion methods showing zone of inhibition (in mm). Where, 'E' = 50% Ethanol; 'C' = Chloroform extract; 'D' = Dichloromethane extract and 'Co' = Control.

## Conclusion

The results suggest that this plant has vast variety of phytochemicals which can be used as effective remedy for various ailments and drug formulations in future either alone or in combination with other suitable agents. The ethnic claims of this plant were also verified by the present study as there was presence of phytochemicals which show these activities.

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## Conflict of interests

Declared None

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