Research Article

Phytochemical Evaluation, Gas Chromatography-Mass Spectroscopy (GC-MS) and Anti-Bacterial Activity Studies from *Crataeva nurvala* Buch Ham

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ABSTRACT

The aim of this study was to analyze the phytochemical compounds and assess *Crataeva nurvala* Buch Ham antibacterial efficacy and Gas chromatography-mass spectrometry (GC-MS) analysis. A range of bacterial strains was tested for antimicrobial activity using different extracts of *Crataeva nurvala* by agar well diffusion technique. *Crataeva nurvala* was found to be rich in alkaloids, saponins, tannins, and resins. The chemical characterization revealed the presence of 20 distinct phytocompounds using GC-MS, and the most predominant compounds were alpha-D-mannopyranoside, methyl, cyclic 2,3:4,6-bis-ethyl boronate (21.17%), followed by hexadecanoic acid, methyl ester (9.91%), and bacteriochlorophyll-c-stearyl (4.41%). The ethanolic extract inhibited the growth of selected bacteria One Gram positive bacterial strain *Staphylococcus aureus* in the range of 35 mm and 30 mm in 50 µl and 100 µl dilution by comparing standard Amikacin 30 mm and one Gram negative bacterial strain Escherichia coli in the range of 35 mm and 20 mm in 50 µl and 100 µl dilution by comparing standard Amikacin 20 mm respectively. The ethanolic extract of *Crataeva nurvala* in both microorganisms Staphylococcus aureus and Escherichia coli showed maximum good inhibition in 50 µl dilution. So, it is proved that the ethanolic extract of *Crataeva nurvala* has good anti-microbial activity.

Keywords: Phytocompounds, GC-MS, Anti-bacterial activity, *Crataeva nurvala*.

INTRODUCTION

Naturally, present phyto chemicals in leaves, fruits, seeds, barks and roots of medicinal plants have a protective and resistant mechanism for various diseases. Phytochemicals contain both primary and secondary compounds[1]. Chlorophyll, proteins and common sugars are considered as primary compounds and terpenoids, alkaloids, flavonoids, saponins and phenolic compounds are appraised as secondary compounds[2]. Terpenoids reveals significant pharmacological action against inflammation, cancer, malaria, viral and bacterial infections[3]. Alkaloids are considered as anaesthetic agents, inhibitors of micro-organisms, antihypertensive effects and possess anti-malarial activities which are mainly found in medicinal plants[4,5].

Crataeva nurvala is commonly known as Varuna. Varuna is one of the best litholytic herbs and has been used throughout the ages for the treatment of urolithiasis and crystalluria. Varuna is mentioned in vedic literature, its therapeutic use being known to ancient Ayurvedic physicians, especially as a blood purifier, to maintain homeostasis. The plant has various synonyms in Ayurvedic scriptures delineating its peculiarities viz. triparna-trifoliate, bilvapatra- leaves resemble to those of bilva (Aegle marmelos). Vrttaphala – fruits, ovoid berries, asmari-ghna-litholytic, tikta- bitter etc. Maharsi Susruta has mentioned varuna as a litholytic agent in treating kapha and vata varieties of asmari (calculi)[6,7].

It is an appetizer, febrifuge, diuretic and litholytic in properties. It is used in diseases like urinary disorders, urinary calculi, blood disorders, worms and tumors. The bark of the tree is an important drug for problem affecting the kidneys and bladder. In Ayurveda, the bark of the Crataeva has been traditionally used to heal kidney stones for more than 3,000 years. Findings of several studies undertaken by contemporary scientists have authenticated that the herb neutralizes the enzyme called glycolate oxidase and this particular effect of the herb lessens the production of oxalates by the body[8].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra[9,10]. Herbal drugs are increasingly used worldwide during the last few decades as seen in the rapidly growing global and national markets of herbal drugs[11]. The workhorse of contemporary plant metabolite profiling is no doubt gas chromatography coupled to mass spectrometry (GC-MS)[12]. Therefore, in this study the preliminary phytochemical screening, GCMS analyses and antimicrobial activity was carried out to profile the chemical constituents in them that are responsible for their antimicrobial activity and other medicinal properties.

MATERIALS AND METHODS

Collection of materials

The bark powder of *Crataeva nurvala* is obtained from 3V products, Avadi, Chennai, Tamilnadu.

Processing of bark powder for extract preparation

Crataeva nurvala bark powders were air-dried at room temperature and extraction was done by cold maceration method. The powder (100 g) was macerated in beaker with 80% 400ml ethanol and allowed to stand for 72 h at room temperature. The beaker is covered with aluminium foil. Extractions continued for 7 days. After 7 days the aluminium foil removed, and the extract was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using hot plate to get a brownish green liquid extract.

The percentage yield of extract and the colour of the extract was noted in Table 1.

Table 1: Percentage yield and the colour of the extract of *Crataeva nurvala* bark powder

Extract	Percentage yield	Colour
Ethanol extract	13.5%	Brownish green

Preliminary Phytochemical Investigation[13-15]

Phytochemical analysis reveals the occurrence of alkaloids, anthracene derivatives, flavonoids, phenolics, phytosterols, saponins, tannins, triperpenoids and volatile oils. (Table 2)

Gas Chromatography-Mass Spectroscopy (GC-MS)

GC-MS analysis of ethanol extract was carried out using the equipment GC Clarus 500 Perkin-Elmer system comprising a AOC–20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: Equipped with a column Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1µm df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as carrier gas at a constant flow rate of 1.20 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature is set at 250 °C, and the ion-source temperature is 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase for 10 °C/min, to 200°C/ min, then 5°C/ min to 280 °C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds, and fragments from 45 to 450 Da. Total GC running time was 36 min[16].

Table 2: Phytochemical analysis of *Crataeva nurvala* bark powder

S.NO	Phyto constituents	Ethanol extract
1	Alkaloids	+
2	Carbohydrates	-
3	Tannins	+
4	Saponins	+
5	Triterpenoids	+
6	Proteins and amino acids	-
7	Phenolic compounds	+
8	Gums and mucilages	-
9	Fixed oil and fats	-
10	Glycosides	+
11	Triterpenes	+
12	Phytosterols	+

+ = presence of active constituents; - = absence of active constituents

Table 3: Compounds identified in *Crataeva nurvala* bark powder of ethanolic extract

S.No	R.T	Name of the compound	Molecular Formula	Molecular Weight
1	4.055	1-(3-butenyloxy)-1-(trimethylsiloxy)ethylene	$C_9H_{18}O_2$	186
2	4.176	Erythro/threo-5-[(benzylamino)methy]-4-tosyl-5-hexen-3-ol	$C_7H_{14}O_3$	146
3	8.524	Propane, 1,1-diethoxy-	$C_{21}H_{27}NO_3S$	373
4	19.957	1,2-benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278
5	21.203	1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester	$C_{12}H_{20}O_7$	276
6	26.303	Benzothiazole, 2-(2hydroxyethylthio)-	$C_9H_9NOS_2$	211
7	26.617	Decanoic acid	$C_{10}H_{20}O_2$	172
8	29.295	1-allyloxy-octa-2,7-diene	$C_{11}H_{18}O$	166
9	29.39	(z)-1-iodo-2,3-epoxy-undec-5-ene	$C_{11}H_{19}IO$	294
10	29.755	Glutaraldehydic acid, 4,4-dimethyl-	$C_7H_{12}O_3$	144
11	34.864	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390
12	35.753	2-decyloxyethanol	$C_{12}H_{26}O_2$	202
13	37.479	1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390
14	38.174	2-decyloxyethanol	$C_{12}H_{26}O_2$	202
15	38.294	di-isodecyl phthalate	$C_{28}H_{46}O_4$	446
16	38.397	di-isodecyl phthalate	$C_{28}H_{46}O_4$	446
17	38.631	1,2-benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390
18	38.968	1,2-benzenedicarboxylic acid, dicyclohexyl ester	$C_{20}H_{26}O_4$	330
19	39.093	1,2-benzenedicarboxylic acid, dicyclohexyl ester	$C_{20}H_{26}O_4$	330
20	39.404	Succinic acid, 2,4,6-trichlorophenyl tetrahydrofurfuryl ester	$C_{15}H_{15}Cl_3O_5$	380

GC-MS Analysis of Samples

The given *Crataeva nurvala* bark powder sample was extracted with ethanol and analysed through GC-MS for identification of different compounds.

Identification of compounds in GC-MS analysis

20 Compounds were identified in *Crataeva nurvala* bark powder by GC-MS analysis. The active principles with their retention time (RT), Molecular weight (MW) and concentration are presented in the compound of various extracts are given in table 3.

Anti-Microbial Activity

Microorganisms used

One Gram positive bacterial strains Staphylococcus aureus and one Gram negative bacterial strains Escherichia coli. All the bacterial strains were obtained and done in Bose clinical laboratories, Madurai District, Tamil Nadu.

The test organisms were prepared by inoculating a loop full of culture in a 5ml of nutrient broth and incubated at 37°C for 14 hours.

***Anti-bacterial activity*[17-19]**

The antimicrobial activities of the extract were evaluated by means of the agar well diffusion assay. The assay was carried out according to the method of Natarajan et al. (2005) with some modifications.

Approximately 25 ml of Muller Hinton Agar (MHA) (Hi Media) were poured into sterile petri dish and allowed to solidify.

About 100 µl of bacterial inoculums were poured then swabbed on the MHA media by using sterile cotton swab. In each of these plates five wells (5mm diameter) were punched into the agar by using sterile cork borer. Then 50 µl and 100 µl dilution of ethanolic extract was separately added into wells and allowed to diffuse at room temperature. Equal volume of DMSO was served as negative control and standard antibiotic Amikacin used as positive control. The plates were incubated for 24 hours at 37°C and the diameter (in mm) of clear zone of growth inhibition was recorded.

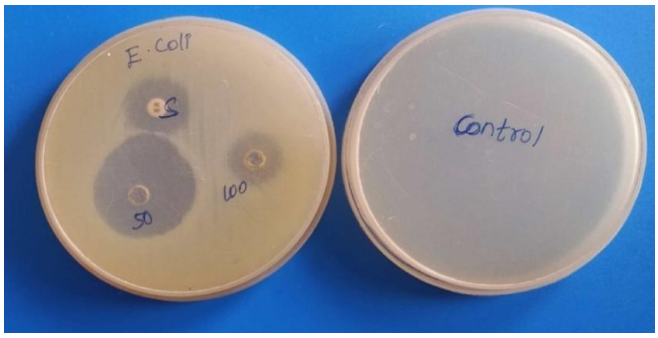

Figure 1: Anti-bacterial activity of Escherichia coli (Gram negative)

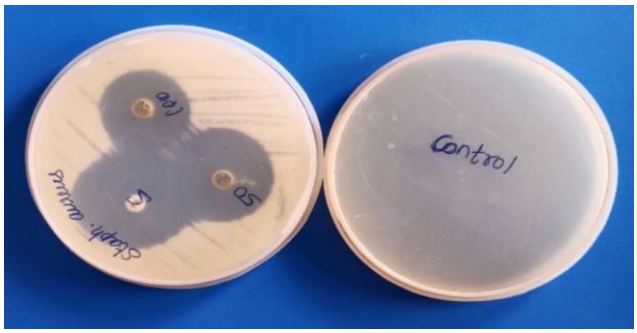

Figure 2: Anti-bacterial activity of *Staphylococcus aureus* (Gram positive)

Table 4: Anti-bacterial activity of ethanol extract of *Crataeva nurvala*

Organisms	Standard disc	50 dilutions	100 dilutions
Escherichia coli	AMIKACIN-20 mm	35 mm	20 mm
Staphylococcus aureus	AMIKACIN-30 mm	35 mm	30 mm

RESULTS AND DISCUSSION

Preliminary Phytochemical Investigation

Phytochemical analysis reveals the occurrence of alkaloids, anthracene derivatives, flavonoids, phenolics, phytosterols, saponins, tannins, triperpenoids and volatile oils.

Gas Chromatography -Mass Spectroscopy (GC-MS)

Identification of compounds in GC-MS analysis

20 Compounds were identified in *Crataeva nurvala* bark powder by GC-MS analysis. The active principles with their retention time (RT), Molecular weight (MW) and concentration are presented in the compound of ethanol extract.

Anti-Microbial Activity

The results of the antimicrobial activity by the agar well diffusion method of *Crataeva nurvala* ethanolic extract. The ethanolic extract inhibited the growth of selected bacteria (One Gram positive bacterial strain Staphylococcus aureus in the range of 35 mm and 30 mm in 50 µl and 100 µl dilution by comparing standard Amikacin 30 mm and one Gram negative bacterial strain Escherichia coli in the range of 35 mm and 20 mm in 50 µl and 100 µl dilution by comparing standard Amikacin 20 mm respectively (Table 3). The ethanolic extract of *Crataeva nurvala* in both microorganisms Staphylococcus aureus and Escherichia coli showed maximum good inhibition in 50 µl dilution. So, it is proved that the ethanolic extract of *Crataeva nurvala* has good anti-microbial activity Fig 1 and 2.

CONCLUSION

Crataeva nurvala is a traditional medicinal plant and represents rich source of compounds possessing antimicrobial properties. Till now, little work has been carried out on their biological properties and hence extensive research is required to explore and identify the potential biological compounds of medicinal importance. The results of the present study revealed that the *Crataeva nurvala* ethanolic extract of bark powder showed the presence of 20 compounds in GC-MS (Gas chromatography -Mass spectroscopy) analysis and also it could be used as powerful antimicrobial agents for the prevention of many diseases. Further study can be extended to check their ant-oxidative properties.

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REFERENCES

1. Vijyalakshmi R, Ravindran R. Preliminary comparative phytochemical screening of root extracts of *Diospyros ferrea* (Wild.) Bakh and Arvalanata (L.) Juss. Ex Schultes. Asian J Plant Sci Res 2012;2:581-7.
2. Gupta MK, Shrivastava AK, Singh AP, Chauhan, Gaur DS, Mishra KK, et al. Comparative study of the *Shiva guggulu* and *Simhanada guggulu* in the management of amavata (rheumatoid arthritis). Int J Appl Pharm 2016;2:5-12.
3. Dinesh Kumar et al., Future journal of pharmaceutical sciences. 2020;113-21.
4. Battacharjee et al., Asian Pacific journal of Tropical Biomedicine. 2012; S1162-S1168.
5. Srikarwar and patil, Asian journal of pharmacy and pharmacology 2020;6(2):119-125.
6. Farnsworth NR. The Role of Ethnopharmacology in Drug Development. In: Bioactive Compounds from Plants (Ciba Foundation Symposium 154), Chadwick D.J. and J. Marsh (Eds.). Wiley, Chichester, UK. p. 2-21.
7. http://logayurveda.com/plant-profiles/204-*Crataeva nurvala*.pdf (accessed jan3, 2012).
8. http://www.herbosophy.com.au/crataeva-*Crataeva nurvala*/ (accessed jan3, 2012.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants. Indian Press. 1918. p. 34-44.
10. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997. p. 609-611.
11. Muyumba NW, Mutombo SC, Sheridan H, Niachtergael A and Duez P. Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications. Talanta Open. 2021;4:1-11. DOI: 10.1016/j.talo.2021.100070
12. Baba H, Kashimawo AJ and Ibe AC. Phytochemical evaluation and GC-MS profiling of the dichloromethane and ethanol extracts of *Ocimum gratissimum* L. and Lasianthera Africana BEAUV. Journal of Phytomedicine and Therapeutics. 2022; 20(2):640-655.
13. PhillipsDR, Rasbery JM, Bartel B, Matsuda SPT. Biosynthetic diversity in plant triterpene cyclization. Current Opinion in Plant Biology 2006; 9: 305–14.
14. Lindsey K, Pullen ML, Topping JF. Importance of plant sterols in pattern formation and hormone signaling. Trends Plant Science. 2003; 8: 521–5.
15. Anjaria Jayvir, Parabia Minoo, Bhatt Gauri and Khamar Ripal. A glossary of selected indigenous medicinal plants of India. 2nd ed. SRISTI Innovations 2002. p. 23.
16. Merlin NJ, Parthasarathy V, Manavala R and Kumaravel S: Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. Pharm Res 2009;1(3):152-56.
17. Natarajan D., John Britto S., Srinivasan K., Nagamurugan N., Mohanasundari C. and Perumal G. Anti-bacterial activity of *Euphorbia fusiformis* – A rare medicinal herb. J. Ethnopharmacol. 2005;102(1):123-126.
18. Valsaraj R., Pushpangadan P., Smit U.W., Adsersen A. and Nyman U. Antimicrobial screening of selected medicinal plants from India, J. Ethnopharmacol. 1997;58:75-83.
19. Raghvendra M.P., Satish S. and Raveesha K.A. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*, a known medicinal plant, my SCIENCE, 2006;1:72-78.