RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2021.52.2.135-141

Qualitative and quantitative analysis (GC-MS) of methanol extract of *Crataeva nurvala* stem bark

K. K. Aathira[1], Bibu John Kariyil[2], G. Dhanusha[1], J. S. Haima[1], S. Sujith[3], M. Shynu[4] and A. R. Nisha[5]
Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651. Kerala Veterinary and Animal Sciences University, India.

Citation: Aathira, K. K., Bibu, J. K., Dhanusha, G., Haima, J. S., Sujith, S., Shynu, M. and Nisha, A. R. 2021. Qualitative and quantitative analysis (GC-MS) of methanol extract of *Crataeva nurvala* stem bark. *J. Vet. Anim. Sci.* **52**(2): 135-141. DOI: https://doi.org/10.51966/jvas.2021.52.2.135-141

Received: 21.12.2020 *Accepted: 20.01.2021* *Published: 01.06.2021*

Abstract

Medicinal plants are precious source of bioactive compounds which possess a range of beneficial properties and serve as the major source of medicine for a large proportion of population across the world. Since ancient times, Crataeva nurvala has been used as a vital herb in Ayurvedic system of medicine. In Unani system of medicine the bark of C. nurvala is used as an appetite stimulant and as an agent to decrease the secretion of bile and phlegm. In the present study, the methanol extract of stem bark of C. nurvala was analysed for preliminary phytochemicals and the chemical profiling of the extract was illustrated using gas chromatography mass spectrometry (GC-MS) analysis. The phytochemical analysis revealed that the plant extract contained alkaloids, steroids and triterpenoids. The GC-MS analysis determined the presence of different compounds of biological importance. The identification and characterisation of the phytoconstituents in the extract could pave the way for the discovery of new drugs for various ailments.

Keywords: *Crataeva nurvala, triterpenes, alkaloids, steroids.*

Medicinal plants are valuable source of naturally active phytochemicals which provide health benefits for humans and animals. These compounds commonly known as secondary plant metabolites have been attributed to have different biological properties which provide protection against various diseases. The analysis of the chemical constituents present in those plant extracts

**Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala*

1. *MVSc Scholar*
2. *Assistant Professor corresponding author: email: bibujohn@kvasu.ac.in Ph: 9895297842*
3. *Assistant Professor*
4. *Associate Professor, Department of Veterinary Biochemistry*
5. *Assistant Professor and Head*

is strategic to comprehend many herbal drugs and their preparations which would further aid in discovering the actual values of folklore remedies.

Crataeva nurvala Buch-Ham., commonly known as Varuna, Neermathalam, Barna Chal, belonging to the family of Capparidacea, is a moderate sized deciduous tree. A variety of medicinal properties have been reported for *C. nurvala* and its stem bark. It has been traditionally used in normalising blood flow, waste elimination, breathing problems, fever, metabolic disorders, joint lubrication and wound healing (Vashist *et al*., 2020). Mekap *et al.* (2011) determined the antiurolithiatic activity of *C. nurvala*. Root and bark extracts of this tree are documented to be laxative, lithotripic and have been found to increase the appetite and biliary secretion (Malini *et al.*, 1995). The ethanol and aqueous extracts of the dried stem bark of *C. nurvala* have been found to possess significant anti-fertility effects in rats (Bhaskar *et al*., 2009). The antidiarrhoeal activity of ethanol extracts of *C. nurvala* stem bark has been reported by Inayathulla *et al*. (2010). *Crataeva nurvala* stem bark extract exhibited antidiabetic activity against alloxan induced diabetic albino rats in the study done by Sikarwar and Patil (2010). Thus, the present study was carried out to evaluate the various phytochemical constituents present in the bark of methanol extract of *C. nurvala* which would be helpful to delineate the various biological activities shown by the stem bark.

Materials and methods

Plant collection and identification

The bark of *Crataeva nurvala* was collected from Valluvanad, Palakkad, Kerala (Figure 1 and 2). The collected plant material was identified and its authenticity was confirmed by Raw Material Herbarium and Museum (RHMD), NISCAIR, New Delhi, India.

Preparation of extracts

Freshly collected bark of *C. nurvala* were cleaned to remove adhering dust and then dried under shade. The dried bark was coarsely powdered using an electric pulveriser and the powder obtained was extracted using a Soxhlet apparatus with methanol at 67°C. The methanol extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature (40°C). The yield of the extract was calculated using the formula: Yield value (%) = Extracts obtained/ Total amount of crude drug × 100, and kept under refrigeration in an airtight container after complete evaporation of the solvent for further use.

Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive compounds using methods described by Harborne (1998).

Tests for detection of steroids

Salkowski's test

Fifty milligrams of the extract were dissolved in 3 mL of chloroform. Few drops of concentrated sulphuric acid were added and the solution was allowed to stand. Formation of red colour directed the presence of steroids.

Liebermann Burchardt test

Fifty milligrams of the extract were mixed with 3 mL of chloroform. To this, five drops of acetic anhydride and 1 mL concentrated sulphuric acid was added along the sides of the test tube. Development of a reddish ring at the junction of two layers confirmed the presence of steroids.

Tests for detection of alkaloids

One gram of the extract was mixed with 5 mL of ammonia and then extracted with an equal volume of chloroform. To this extract, 5 mL of dilute hydrochloric acid was added. The acid layer obtained was further tested with the following reagents for the presence of alkaloids.

Dragendorff's test

Eight drops of Dragendorff's reagent was mixed with 1 mL of acid extract. Development of a reddish brown precipitate indicated the presence of alkaloids.

Fig. 1. Herbarium of *Crataeva nurvala* Buch-Ham

Fig. 2. Bark of *Crataeva nurvala* Buch-Ham.

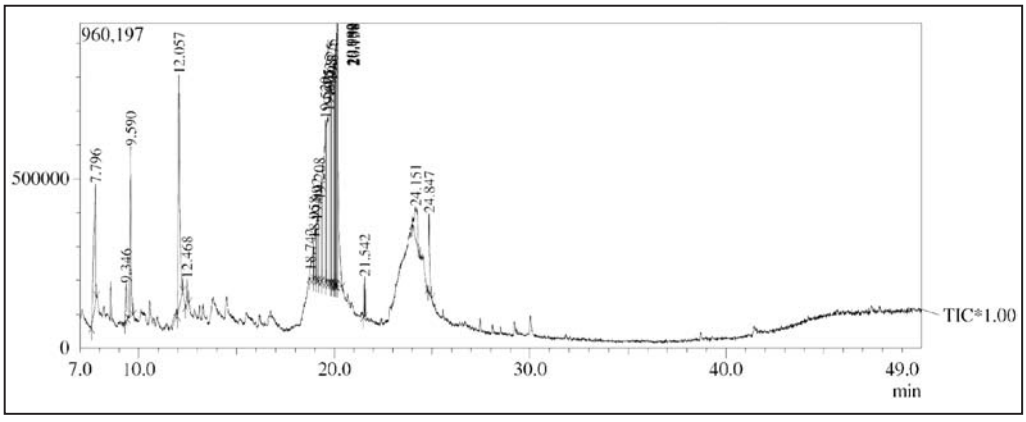

Fig. 3. GC-MS chromatogram of methanol extract of *C. nurvala* stem bark

Mayer's test

To 1 mL of the acid layer, eight drops of Mayer's reagent were added. Development of a cream coloured precipitate indicated the presence of alkaloids.

Wagner's test

One millilitre of Wagner's reagent was added to 1 mL of the extract. Development of reddish brown colour precipitate indicated the presence of alkaloids.

Hager's test

To 1 mL of the acid extract, eight drops of Hager's reagent were mixed. Formation of yellow precipitate specified the presence of alkaloids.

Tests for detection of glycosides

Benedict's test

Approximately 50 mg of the extract was mixed with 1 mL of water and then 5 mL of Benedict's reagent was added to it. Formation of brown or red precipitate indicated the presence of reducing sugars.

Test for detection of phenolic compounds

Ferric Chloride Test

Five milligrams of the extract was dissolved in 1 mL of water and five drops of 10 per cent ferric chloride were added to it. Development of bluish black colour specified the presence of phenols.

Tests for detection of tannins

Ferric chloride test

Treated two milligrams of the extract with 3 mL of one per cent ferric chloride solution. Development of a blue-black or a brownish green colouration showed the presence of tannins.

Tests for detection of flavonoids

Ferric chloride test

Treated 2 mL of the methanol extract (0.5 gram extract in 10 mL methanol) with four drops of neutral ferric chloride solution. Formation of green colour indicated the presence of flavonoids.

Test for detection of diterpenes

About five milligrams extract was mixed with 3 mL of five per cent copper acetate solution. Formation of green colour showed the existence of diterpenes.

Tests for detection of triterpenes

Salkowski test

Mixed 3 mL of chloroform to three milligrams of extract and it was shaken with 3 mL concentrated sulphuric acid. Development of yellow colour in the lower layer on standing indicated the presence of triterpenes.

Tests for detection of saponins

Froth test

Approximately 200 mg of the extract was shaken with 5 mL of water. Continuation of foam produced for ten minutes designated the occurrence of saponins.

GC-MS Analysis

The active phytochemical principles of methanol extract of *C. nurvala* was analysed using GC-MS system of Centre for Analytical Instrumentation- Kerala (CAI-K), Kerala Forest Research Institute (KFRI), Peechi, Kerala. The GC-MS analysis was carried out on Gas chromatography Mass Spectrometer (Shimadzu GC-MS, Japan, QP2010SE) with a mass range of 1.5- 1000 m/z. Helium at a flow rate of 1 mL/ min was used as the carrier gas. The oven temperature was maintained at 80°C for 4 min and then increased to 280°C in 6 minutes. The injector temperature was 260°C and total analysis time was 50 minutes. Aliquot of the extract (0.4 µL) was injected into the chromatographic column after obtaining a clear baseline. The interpretation of the mass spectrum of GC-MS was guided using the database of the National Institute of Standards and Technology (NIST 11) and WILEY 8. The spectrum of the unknown compounds was related with the spectrum of the known compounds. The name and molecular weight of the compounds of the tested materials were ascertained.

Results and Discussion

Yield of the extract

The methanol extract of *C. nurvala* yielded 12.93 per cent with reference to starting dry material.

Qualitative phytochemical analysis

The qualitative phytochemical screening of methanol extract of stem bark of *C. nurvala* showed the presence of steroids, triterpenoids and alkaloids. Phytochemical screening of methanol extract of stem bark of *C. nurvala* revealed the presence of steroid and terpenoids as well as alkaloids, phenolics, flavanoids, tannins and saponins (Hade *et al.,* 2016) which supported our results. Sodipo *et al.* (2000) have reported that alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Huang *et al.* (2016) isolated six phytosteroids and nine known triterpenoids from the leaves of *Chisocheton cumingianus* in which chisopanoids E and F exhibited potent cytotoxicities towards MCF-7 with IC_{50} values of 3.24 ± 1.39 and 8.85 ± 4.73 µM, and were further proved to prevent the cell proliferation, mainly by inducing apoptosis. Haque *et al.* (2008) isolated two terpenoids, phragmalin triacetate and lupeol from ethyl acetate extract of stem bark of *C. nurvula* by chromatographic

techniques. Jain *et al.* (2016) suggested that terpenoids were capable of inhibiting NFκB through different mechanisms. Khatun *et al.* (2015) evaluated the antioxidant, anthelmintic, antimicrobial and phytochemical assessment of ethanolic extract of *C. nurvala* leaves and displayed the presence of alkaloids, flavonoids, reducing sugar, saponins, steroids, tannins. The above mentioned phyto constituents are described to exhibit various pharmacological activities.

GC- MS analysis

The results of GC-MS analysis of methanol extract revealed the presence of twenty-one compounds. The GC-MS chromatogram of twenty-one compounds is depicted in Figure 3. Thymine, 3-hydroxy-2,3-dihydromaltol, 5-hydroxymethylfurfural, n-methyl-3-hydroxymethyl pyrrolidine-2-one, cytidine, methyl pentofuranoside, undecane, 6,6-dideutero-5-methyl-, 2,4-ditert-butylphenol and 3-deoxy-d-mannoic lactone were the major compounds.

Balamurugan *et al.* (2019) have done the chemical profiling of methanol bark extract of *C. nurvala* using GC-MS technique. The study revealed the presence of 8 components such as lup-20 (29)-en-3ol, 2-hydroxy-4methoxybenzaldehyde, methoprene, 1'-acetonaphthone, 1, 2-bis (Trimethylsilyl) benzene, pivalate, cyclotrisiloxane, limonen-6-ol and 4-hexadecen-6-yne.

The recognized major compounds in our study possess some significant biological activities for future drug development. Zhao *et al.* (2013) showed that 5-hydroxymethylfurfural (5-HMF) induced apoptosis and G0/G1 cell cycle arrest in human melanoma A375 cells. Takuli *et al.* (2020) elucidated the antioxidant and antibacterial activity of *Woodwardia unigemmata* (Makino) along with chemical characterization which revealed the presence of 3-hydroxy-2,3-dihydromaltol in GC-MS analysis. Azizi *et al.* (2006) performed fast gas chromatography/ time of flight mass spectrometry (TOF-GCMS) which identified N-methyl-3-hydroxymethylpyrrolidin-2-one from the oil extract of *Pithecellobium jiringan* jack seeds which was found to abolish excess free radicals and counteract oxidative damage. Su *et al*. (2005) evaluated the antioxidant activity of methanol extract of *Morinda citrifolia* (Noni) fruits and the purification of its butanol soluble partition of methanol extract contained isolates like cytidine. Shaheed *et al.* (2018) identified methyl pentofuranoside, also known as alpha-d-mannofuranoside, from methanolic fenugreek seed extract and determined its antibacterial activity against *Streptococcus agalactiae, Escherichia coli, Enterococcus cloacae* and *Proteus mirabillis.* Gas chromatography mass spectroscopic analysis exhibited the presence of undecane,6,6-dideutero-5-methyl- in *Nigella sativa*, *Allium sativum*, *Propolis* and *Olea europaea* mixture which was depicted as antibacterial and antifungal agent (Bintang *et al.*, 2018). Chuah *et al*. (2015) suggested that 2,4-di-tert-butylphenol induced oxidative stress through the generation of reactive oxygen species, which cause lipid peroxidation and membrane damage in root tissues and chloroplast in leaf tissues, thus leading to increased levels of antioxidant enzymes. Shobana *et al.* (2009) in their study identified compounds such as 3-deoxy-d-mannoic lactone and thymine from two varieties of garlic (ophioscordon and sativum) which was found to possess antibacterial activity against enteric pathogens. The aforesaid isolated compounds from the methanol extract of *C. nurvala* stem bark seemed to own the reported biological activity and further study of these phytoconstituents may demonstrate the medicinal importance in future. The biological activities of other compounds have not been reported so far and more study of these phytoconstituents might validate the significant medicinal features in forthcoming.

References

Azizi, C.Y.M., Norulaini, N.A. N., Setianto, W.B. and Omar, A. K. M. Supercritical carbon dioxide extraction of constituents of *Pithecellobium jiringan* seeds and their identification using time of flight gas spectrometry. *Proceedings of the 1st International Conference on Natural Resources Engineering & Technology*; 24th-25th July 2006. Putrajaya, Malaysia. pp. 616-625.

Balamurugan, V., Revathi, E., Kamalakkannan, J. and Sundaresan, A. 2019. Anticancer activity of methanol extract of *Crataeva nurvala* in HeLa cell Line*. Int. J. Cell Biol. Cell. Processes.* **5:** 9-22.

Bhaskar, V.H., Profulla, K.M., Balakrishnan, B.R., Balakrishnan, N. and Sangameswaran, B. 2009. Evaluation of the anti-fertility activity of stem bark of *Crataeva nurvala* buch-hum. *Afr. J. Biotechnol.* **22**: 6453-6456.

Bintang, M., Pasaribu, F.H., Safira, U.M. and Sidhartha, T. 2018. Identification of bioactive compound from *Nigella sativa*, *Allium sativum*, *propolis* and *oleaeuropaea* mixture as antibacterial and antifungal agent. *Earth Environ. Sci*. **196**: 12041p.

Chuah, T.S., Norhafizah, M.Z. and Ismail, B.S. 2015. Evaluation of the biochemical and physiological activity of the natural compound, 2, 4-ditert-butylphenol on weeds. *Crop Pasture Sci*. **2**: 214-223.

Hade, S.N., Joshi, P.A., Pilley, H.H., Wadegaonkar, V.P. and Wadegaonkar, P. A. 2016. Evaluation of *Crataeva nurvala* extracts as antioxidant, antiproteolytic and cytotoxic against hepato-carcinoma and mouse melanoma cell lines. *J. Appl. Pharm. Sci.* **6**: 189-196.

Haque, M.E., Islam, M.N., Gupta, D.D., Hossain, M., Shekhar, H.U. and Shibib, B.A. 2008. Triterpenoids from the stem bark of *Crataeva nurvala*. *Dhaka Univ. J. Pharm. Sci*.**7**: 71-74.

Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. (3rd Ed.). Chapman and Hall, London. 320p.

Huang, S.S., Jian, K.L., Li, R.J., Kong, L.Y. and Yang, M.H. 2016. Phytosteroids and triterpenoids with potent cytotoxicities from the leaves of *Chisocheton cumingianus*. *RSC Adv.* **8**: 6320-6328.

Inayathulla, S.W.R., Asif, K. and Mukesh, S. 2010. Ethanolic extract of *Crataeva nurvala* root bark in experimental animals. *Int. J. Pharm. Pharm. Sci*. **2**: 158-61.

Jain, H., Dhingra, N., Narsinghani, T. and Sharma, R. 2016. Insights into the mechanism of natural terpenoids as NF-κB inhibitors: An overview on their anticancer potential. *J. Clin. Exp. Oncol.* **38**: 158-168.

Khatun, F., Mahfuz-E-Alam, M., Tithi, N.S., Nasrin, N. and Asaduzzaman, M. 2015. Evaluation of phytochemical, antioxidant, anthelmintic and antimicrobial properties of *Crataeva nurvala* Buch. Ham. leaves. *Int. J. Pharm. Sci. Res*. **4**: p.1422.

Malini, M.M., Baskar, R. and Varalakshmi, P. 1995. Effect of lupeol, a pentacyclic triterpene, on urinary enzymes in hyperoxaluric rats. *Jpn. J. Med. Sci. Biol.* **1**: 211-220.

Mekap, S.K., Mishra, S., Sahoo, S. and Panda, P.K. 2011. Antiurolithiatic activity of *Crataeva magna* Lour. bark. *Indian J. Nat. Prod. Resour*. **1**: 28-33

Shaheed, K.A., Alsirraj, M.A., Allaith, S.A., Noori, N.A., Obaid, M.H., Mouhsan, Z.M. and Swedan, S.S. 2018. The biological activities of seeds extracts for fenugreek and black cumin and its inhibitory influences towards some pathogens. Iraq Med. J. 2: 46-50.

Shobana, S., Vidhya, V.G. and Ramya, M. 2009. Antibacterial activity of garlic varieties (ophioscordon and sativum) on enteric pathogens. *Curr. Res. J. Biol. Sci.* **3**: 123-126.

Sikarwar, M.S. and Patil, M.B. 2010. Antidiabetic activity of *Crateva nurvala* stem bark extracts in alloxan-induced diabetic rats. *J. Pharm. Bioallied Sci.* **1**: p.18.

Sodipo, O.A., Akinniyi, J.A. and Ogunbameru, J.V. 2000. Studies on certain characteristics of extracts of bark of

Pausinystalia johimbe and *Pausinystalia macroceras* (K Schum) Pierre ex Beille. *Glob. J. Pure and Applied Sci.* **1**: 83-88.

Su, B.N., Pawlus, A.D., Jung, H.A., Keller, W.J., McLaughlin, J.L. and Kinghorn, A.D. 2005. Chemical constituents of the fruits of *Morinda citrifolia* (Noni) and their antioxidant activity. *J. Nat. Prod.* **4**: 592-595.

Takuli, P., Khulbe, K., Kumar, P., Parki, A., Syed, A. and Elgorban, A.M. 2020. Phytochemical profiling, antioxidant and antibacterial efficacy of a native Himalayan Fern: *Woodwardia unigemmata* (Makino) Nakai. *Saudi J. Biol. Sci.* **8**: 1961-1967.

Vashist, S., Choudhary, M., Shammi Rajpal, D.S. and Budhwar, V. 2020. Varuna (Buch. Ham.): A brief review on Phytochemistry, *Crataeva nurvala* pharmacological profile and uses in various ailments. *Asian J. Pharm. Pharmacol.* **2**: 119-125.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z. and Chen, T. 2013. *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *J. Agric. Food Chem*. **44**: 10604-10611. ■