

EVALUATION OF ANTIBACTERIAL AND ANTHELMINTIC ACTIVITY OF ROOT EXTRACT OF *CRATAEVA NURVALA*

Rajesh Kamath¹, Devraj Shetty¹, Pratibha Bhat², A R Shabaraya¹, Karunakar Hegde^{*1}

¹Srinivas College of Pharmacy, Valachil, Post- Parangepete, Mangalore- 574 143, Karnataka, India.

Government High School Sajipanadu, Post- Sajipanadu, Bantwala, Mangalore- 574 231, Karnataka, India.

Summary

The ethanol extract of the roots of *Crataeva nurvala* (EECN) was investigated for antibacterial and anthelmintic activity. The extract was assayed for antimicrobial activity against various microorganisms such as *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* by agar well diffusion method. The extract showed varies levels of antimicrobial activity on different test microorganisms. The extract was also assayed for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-50 mg/ml) of root extract were tested in the bioassay. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant antibacterial and anthelmintic activity at highest concentration of 50 mg/ml. Future studies are in process to isolate the active principles responsible for the activity.

Key words: Anthelmintic, Antibacterial, *Crataeva nurvala*, Root extract

Address for correspondence:

Mr. Karunakar Hegde
Department of Pharmacology
Srinivas College of Pharmacy
Valachil, Post- Parangepete
Mangalore- 574 143, Karnataka, India.
E-mail: khegde_sh2003@yahoo.co.in

Introduction

Control of microorganisms is critical for the prevention and treatment of diseases. In recent years many microbial diseases like chicken guniya, dengue fever, malaria, AIDS, etc. have become challenging to the modern medical world. A majority of synthetic antibiotics controls the growth and development of microorganisms effectively, but they are highly toxic at their optimum dosage level. Among many proposed strategies, a good understanding of plants offers the potential of developing potent broad spectrum antibiotics.

Worm infestation, is one more prevalent disease and one of the most serious public health problems in the world. Hundreds of millions if not billions of human infections by helminthes exist worldwide and with increased world travel and immigration from the developing countries (1). Modern medicines are gaining less attention due to their limited availability and affordability in human intestinal helminthiasis. Thus, most of the world's population depends to a greater extent on traditional medical remedies.

Crataeva nurvala (Family: Capparidaceae) is a small tree with a much branched head. Leaves are deciduous 3 foliolate; petioles 3.8-7.6 cm long; leaflets are ovate, lanceolate or obovate, acute or acuminate. Fruit a globose or ovoid, woody, smooth or scurfy berry, on the thickened gynophores. The bark is grayish, smooth, vertically cracked. The plant is well distributed almost all over India and Burma, wild or cultivated (2). Often found in the vicinity of temples in Central India, Bengal and Assam. Its bark is hot, bitter at first and then sweet sharp taste, easy to digest, stomachic, laxative, antilithic, anthelmintic, expectorant and antipyretic. Researches shown that its bark contains saponins which are especially useful in urinary complaints such as kidney and bladder stones (2, 3). Critical review of literature revealed scanty information on antibacterial and anthelmintic activity of *C. nurvala*. Thus, the present investigation has been carried to investigate antibacterial and anthelmintic activity ethanol extract of roots of *Crataeva nurvala*.

Materials and Methods

Plant materials and preparation of the extract

The fresh roots of *Crataeva nurvala* were collected from Western Ghats region near Gudnya, Mangalore, Karnataka and authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. The roots were chopped into thin slices and shade dried. Dried roots were crushed to powder and soaked with 70% ethanol for 4 days. After that, the total extract was filtered and dried in rotary flash evaporator. The yield was 9% of the dried weight. The extract was formulated as 10, 20 and 50 mg/mL solutions in DMF (dimethyl formamide) for anthelmintic activity and in 10% DMSO (dimethyl sulfoxide) for antibacterial activity.

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline Pharmaceutical Limited, Bangalore) and Rifampicin (Ranbaxy Laboratories, New Delhi) was used during the experimental protocol.

Phytochemical screening

Freshly prepared hydro ethanolic extract of the roots of *Craetava nurvala* (EECN) was subjected to preliminary phytochemical screening for detection of major chemical constituents (4).

Antibacterial activity

The antibacterial efficacy of ethanol extract of root bark of *C. nurvala* (EECN) was tested against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* by agar well diffusion method (5). Briefly, 24 hours old broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6mm with the help of sterile borer. The standard drug (Rifampicin 1mg/ml) and control (10% DMSO) and different concentrations of extract were added to respectively labeled wells. The plates were incubated at 37 °C for 24 hours in incubator and the zone of inhibition was recorded. Experiment was carried out thrice and average reading was noted.

Anthelmintic activity

The ethanol extract of root bark of *C. nurvala* (EECN) was investigated for its anthelmintic activity against earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (25, 50 and 100 mg/mL) of the extract was tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. The anthelmintic activity was carried as per the method of Ajaiyeoba et al (6) with minor modifications. The anthelmintic activity was evaluated on the adult Indian earth worm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (7-9). Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 5-7 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Use of *Raillietina spiralis* and *Ascaridia galli* as a suitable model for screening of anthelmintic drug was advocated earlier (10, 11).

Test samples of the extract was prepared at the concentrations, 10, 20 and 50 mg/ml in DMF and six worms i.e. *Pheretima posthuma*, *Raillietina spiralis* and *Ascaridia galli* of approximately equal size (same type) were placed in each 9 cm petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and DMF as control. This procedure was adopted for all three different types of worms (12-15). All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). All the results were shown in Table.2 and expressed as a mean \pm SEM of six worms in each group.

Table 1. Antibacterial activity of ethanol extract of the roots of *Craetava nurvala* (EECN)

Group	Treatment	Concentration (mg)	Zone of Inhibition (cm)			
			<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
I	DMSO	-	-	-	-	-
II	EECN	10	8	9.5	11	9
III	EECN	20	12	18	22	14
IV	EECN	50	28	26	24	23
V	Rifampicin	1	35	34	36	37

Table 2. Anthelmintic activity of ethanol extract of the roots of *Craetava nurvala* (EECN)

Group	Treatment	Concentration (mg/ml)	<i>Pheretima posthuma</i> (Earthworm)		<i>Raillietina spiralis</i> (Tapeworm)		<i>Ascaridia Galli</i> (Roundworm)	
			P	D	P	D	P	D
I	DMF	-	-	-	-	-	-	-
II	EECN	10	31±1.99	49±1.25	28±0.64	51±1.52	48±1.09	64±1.82
III	EECN	20	22±0.78	34±0.91	19±0.69	36±1.26	35± 1.01	49±1.10
IV	EECN	50	11±0.84	18±0.64	09±0.34	19±0.67	18±0.48	25±0.86
V	PZC	10	09±0.23	12±0.68	08±0.18	10±0.95	9±0.29	15±0.59

Each value represents mean ± SEM (n=6). DMF: Di-methyl-formamide, PZC: Piperazine citrate, P: Time taken for paralysis of worms (min), D: Time taken for death of worms (min).

Results and Discussion

In this study, the extract has shown inhibition of test bacteria in a concentration dependent manner. Among bacteria, *S. aureus* was found to be more susceptible to extract followed by *P. vulgaris*, *E. coli* and *P. aeruginosa*. Standard antibiotic caused more inhibition of test bacteria than methanol extract. No inhibition of test bacteria was observed in case of control i.e., 10% DMSO. It appears that overall the bacteria were found to be sensitive to extract (Table 1).

From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. The ethanol extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for all three types of worms. However, extract exhibited prominent activity at lower concentration (10 mg/ml) against all three types of worms. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate, which was more potent than the seed extract activity (Table 2).

Preliminary phytochemical screening of extract revealed the presence of flavonoids, terpenes and other phenolic compounds. Antimicrobial activities of tannins, flavonoids, saponins and terpenoids have been documented (16-18). The reasons for the antibacterial activity could be

that the components from the plant active against microorganisms. Phenolic compounds show anthelmintic activity (19). It is possible that phenolic contents in the extract of roots of *C. nurvala* produced similar effects.

From the above results, it is concluded that *Crataeva nurvala* roots used by tribals traditionally to treat infectious and intestinal diseases, showed promising antibacterial and anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as antibacterial and anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for these activities.

Acknowledgement

The authors are thankful to the authorities of A Shama Rao Foundation Mangalore, Karnataka, India for the facilities.

References

1. Williams DA, Lemke TL. Parasitic Infections -Helminthes In: Foye's Principal of Medicinal Chemistry. 5th ed. New York: Lippincott William and Wilkins; 2002.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. II, (Lalit Mohan Basu, Allahabad), 2003;191-193.
3. Nadkarni, KM., Indian Materia Medica, vol.1, Popular Prakashan Pvt. Ltd, Mumbai, 2005; 387-388.
4. Harborne JB. Phytochemical methods, A Guide to modern techniques of plant analysis, 2nd ed. (Chapman and Hall, London) 1984,84.
5. Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissio M, Sokmen A. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry 2004;84:519-525.
6. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* extract. Pharmaceutical Biology 2001;39:217-220.
7. Vigar Z. Atlas of Medical Parasitology. Ed 2nd, P.G. Publishing House, Singapore, 1984:242.
8. Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of *Evolvulus alsinoides* Linn. for anthelmintic and antimicrobial activities. Journal of Natural Remedies 2002;2:182-185.
9. Shivkumar YM, Kumar VL. Anthelmintic activity of latex of *Calotropis procera*. Pharmaceutical Biology 2003;41:263-265.
10. Kaushik RK, Katiyar JC, Sen AB. Studies on the mode of action of anthelmintics with *Ascaridia galli* as a test parasite. Indian Journal of Medical Research 1974;64:1367-1375.
11. Yadav AK. Anthelmintic activity of *Gynura angulosa* against *Trichinella spiralis* infections in mice. Pharmacologyonline 2006;2:299-306

12. Lal J, Chandra S, Raviprakash V, Sabir M. In vitro anthelmintic action of some indigenous medicinal plants on *Ascardia galli* worms. Indian Journal of Physiology and Pharmacology 1976;20:64-68.
13. Mali RG, Shailaja Mahajan, Patil KS. Anthelmintic activity of root bark of *Capparis spinosa*. Indian Journal of Natural Products 2005;21 50-51.
14. Mali RG, Wadekar RR. In Vitro anthelmintic activity of *Baliospermum montanum* Muell. arg roots. Indian Journal of Pharmaceutical Sciences 2008;70:131-133.
15. Gbolade AA, Adeyemi AA. Investigation of in vitro anthelmintic activities of *Pycnanthus angeolensis* and *Sphenocentrum jollyanum*. Fitoterapia 2008;79:200-222.
16. Doss A, Mubarack HM, Dhanabalan R. Pharmacological importance of *Solanum trilobatum*. Indian Journal of Science and Technology 2009;2(2):41-43.
17. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a by product of the essential oil industry. Journal of Applied Microbiology 2007;103(6):2056-2064.
18. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzyska M. Antimicrobial activity of saponins from *Medicago* sp.: structure-activity relationship. Phytotherapy Research 2006;20(6):454-457.
19. Kane RS, Mohite KS, Shete SJ. Antihelmintic activity of aqueous and methanolic extracts of *euphorbia thymifolia* linn. International Journal of Pharmaceutical Technology and Research 2009; 1:666-669.