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Phytochemical profile and comparative antimicrobial and wormicidal activity of ethanol and methanol extracts of *Coscinium fenestratum* Colebr. and *Nardostachys jatamansi* DC

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ABSTRACT

The present study was carried to investigate phytochemical profile, antimicrobial and wormicidal activity of ethanol and methanol extracts of *Nardostachys jatamansi* DC and *Coscinium fenestratum* Colebr obtained by Soxhlet extraction process. Agar well diffusion method was employed to check the efficacy of extracts against bacteria and fungi. Wormicidal activity was assessed by determining paralysis and death time in earthworm model. The solvent extracts of the selected plants showed the presence of various phytochemical groups. A marked antibacterial activity was observed in case of *C. fenestratum* extracts followed by *N. jatamansi*. The extracts of *N. jatamansi* were found to inhibit test fungi to more extent than *C. fenestratum*. A dose dependent wormicidal activity was observed. Among extracts tested, *N. jatamansi* was found to be more efficient in terms of its activity as compared to *C. fenestratum*. the antimicrobial and wormicidal activity of extracts could be mainly attributed to the presence of various phytoconstituents.

KEYWORDS

Nordostachys jatamansi DC;
Coscinium fenestratum Colebr;
Soxhlet extraction;
Phytochemical profile;
Agar well diffusion method;
Wormicidal activity.

INTRODUCTION

Medicinal plants are important elements of traditional medicine in virtually all cultures. Medicinal plants which have been used by human being to treat common infectious diseases are important elements to traditional medicine[1]. *N. jatamansi* (Family Valerianaceae), a critically endangered rhizome-bearing medicinal plant, is restricted to specialized habitats in high altitudes of the Himalaya. The underground part of *N. jatamansi* is used in over 26 Ayurvedic preparations. The root is also used for treatment of heart disease, high blood pressure and insomnia. The root and rhizome contain active compounds with carminative, sedative, antispasmodic and tranquilizing properties[2]. *Coscinium fenestratum* (family Menispermaceae) is a critically endagered dioecious medicinal liana found in Western ghats of India. The stem of the plant is used

in curing several diseases and disorders like diabetes, wounds and ulcers, fever, jaundice, snake bite, piles etc in ethnomedicine. The chief constituent of *Coscinium* is the yellow crystalline alkaloid, berberine[3]. In this study, the ethanol and methanol extract of *N. jatamansi* and *C. fenestratum* were screened for antimicrobial and wormicidal efficacy. The objective of the present investigation was to scientifically validate the folklore use of these two plants in treatment of various kinds of illness.

MATERIALS AND METHODS

Collection of plant material

The dried rhizome of *N. jatamansi* and dried stem of *C. fenestratum* were collected from local shop of Udupi, Karnataka, authenticated to their identity in Dept. of Botany and voucher specimen was deposited for future reference.

Extraction and phytochemical analysis of plant materials

The dried plant materials were powdered mechanically. About 150g of powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol and ethanol solvents separately for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator[4]. The solvent extracts were subjected to preliminary phytochemical analysis[5].

Screening for antibacterial activity

The pure cultures of Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus cereus* and Gram negative bacteria namely *Escherichia coli* and *Salmonella typhi*, obtained from Dept. of Microbiology, were screened for their sensitivity to ethanol and methanol extracts by Agar well diffusion method[6]. In this method, 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF), Standard (Chloramphenicol, 1mg/ml) and Control (DMF) were added into respectively labeled wells. The plates were allowed to stand for about half an hour and then incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded.

Screening for antifungal activity

The antifungal activity of methanol extracts was tested against two species of the genus *Aspergillus* namely *A. niger, A. nidulans* and *A. flavus*. The suspension of spores was prepared in 0.85% sterile normal saline containing 0.01% Tween 80 detergent[7]. The test fungi were screened for their sensitivity by Agar well diffusion method[6]. The spore suspension of test fungi were swabbed uniformly on solidified sterile Sabouraud's dextrose agar plates using sterile cotton swab. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF) and DMF (control) were added into respectively labeled wells. The plates were incubated at room temperature for 72 hours in upright position. After incubation, the diameter of zone of inhibition was recorded.

Screening for wormicidal activity

The assay was performed on adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Indian adult earthworms (*Pheretima pasthuma*) collected from the local earthworm breeder in the outskirts of Shivamogga city were used for the Anthelmintic study. Equal sized (8 ± 1 cm) worms were selected for the study. The worms were washed with normal saline to remove all the extraneous matter. Different concentrations of ethanolic and methanolic extracts namely 50 and 100mg/ml were prepared in normal saline (0.85%) were prepared in normal saline and poured into respective labeled Petri plates (50 ml in each plate). Six worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors[8]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased[9].

RESULTS AND DISCUSSION

TABLE 1 shows the presence of phytochemical groups present in the solvent extracts of selected plants. The EtOH extract of *N. jatamansi* revealed the presence of alkaloids, steroids, flavonoids and tannins while saponins and steroids were not detected. The MeOH of *N. jatamansi* showed the presence of alkaloids, steroids and tannins only. The EtOH extract of *C. fenestratum* showed the presence of all groups except terpenoids while the MeOH of *C. fenestratum* showed the presence of only alkaloids, steroids and flavonoids.

TABLE 1 : Phytochemical profile of methanol and ethanol extracts of selected plants

Phytochemical group	*Coscinium fenestratum*		*Nordostachys jatamansi*	
	EtOH	MeOH	EtOH	MeOH
Alkaloids	+	+	+	+
Terpenoids	ND	ND	ND	ND
Saponins	+	ND	ND	ND
Steroids	+	+	+	+
Flavonoids	+	+	+	ND
Tannins	+	ND	+	+

'+' detected'ND' Not detected

The result of antibacterial activity of EtOH and MeOH extracts of *C. fenestratum* and *N. jatamansi* shows that the EtOH extracts of both the plants were more potent in their antibacterial activity against bacteria causing food poisoning (TABLE 2). Among extracts tested, *C. fenestratum* was found to more potent than *N. jatamansi*. The inhibition recorded by *C. fenestratum* was found to be greater even than the zone of inhibition produced by Standard drug. In case of control, no inhibition of bacteria was observed. Another important observation made was that the Gram positive bacteria were more affected than Gram negative bacteria.

TABLE 2 : Antibacterial activity of methanol and ethanol extracts of selected plants against bacteria causing food poisoning

Treatment	Zone of inhibition in cm			
	S. typhi	*E. coli*	*S. aureus*	*B. cereus*
C. fenestratum EtOH	2.7	3.0	3.8	3.7
C. fenestratum MeOH	2.6	2.8	3.2	3.6
N. Jatamansi EtOH	1.7	1.8	2.0	2.4
N. Jatamansi MeOH	1.6	1.7	2.2	2.4
Standard (Chloramphenical)	2.5	2.6	2.4	2.4

Results are average of three trials

N. jatamansi was found to inhibit test fungi to more extent than *C. fenestratum*. Among extracts tested, the ethanol extract of *N. jatamansi* was more inhibitory than methanol extract except in case of *A. niger*. In case of *C. fenestratum*, more inhibition of fungi was recorded in case of ethanol extract as compared to methanol extract. Ethanol extracts of *N. jatamansi* and *C. fenestratum* were almost similar in their effect (TABLE 3).

TABLE 3 : Antifungal activity of methanol and ethanol extracts of selected plants against *Aspergillus* species

Treatment	Zone of inhibition in cm		
	A. niger	*A. nidulans*	*A. flavus*
C. fenestratum EtOH	1.8	1.8	1.7
C. fenestratum MeOH	1.5	1.5	1.6
N. jatamansi EtOH	1.8	1.9	1.7
N. jatamansi MeOH	2.7	1.7	1.6

Results are average of three trials

TABLE 4 shows wormicidal potency of solvent extracts and from the table, it is clear that both the extracts are very superior in their effect in terms of paralysis and death of worms. Among two concentrations tested, 100mg/ml was more effective suggesting concentration dependent activity of extracts tested. Ethanol extract was found to be more effective in terms of less time taken to cause paralysis and death of worms.

TABLE 4 : Anthelmintic activity of methanol and ethanol extracts of selected plants

Treatment	Concentration in mg	Average time in minutes	
		Paralysis time	Death time
Control	-	-	-
DMF	-	25	65
C. fenestratum EtOH	50	12	38
	100	09	23
C. fenestratum MeOH	50	13	41
	100	10	26
N. jatamansi EtOH	50	12	29
	100	08	18
N. jatamansi MeOH	50	11	31
	100	09	19

Results are average of three trials

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In case of 100mg/ml concentration of both the extracts, the average death time was around 25 minutes and less. Among extracts tested, *N. jatamansi* was found to be more efficient in terms of its activity as compared to *C. fenestratum*.

The higher resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell[10]. Similarly, our results indicated that the antibacterial activities of the extracts were more pronounced on Gram positive than on Gram-negative bacteria. Literature pertaining to antimicrobial activity of *N. jatamansi* is very scarce. Antimicrobial activity of ethanol, ethyl acetate and hexane extracts of *N. jatamansi* roots were studied *in vitro* against gram positive bacteria, gram negative bacteria and fungi. Ethanolic root extract exhibited maximum antimicrobial activity against all the tested bacteria and fungi, at concentrations of 5, 10 and 20 mg/ml as compared to ethyl acetate and hexane extract, which did not show marked activity. Antimicrobial activity was compared with the activities of standard antibacterial and antifungal drugs, namely Ampicillin and Nystatin, respectively. The minimum inhibitory concentrations were between 0.5-1 mg/ml against all the microorganisms[11]. A significant reduction in growth of bacteria and fungi was observed by using the steam distillate of *N. jatamansi*[12]. The *C. fenestratum* extract was found to produce strong inhibition zones against *Propionibacterium acnes* and Phytochemical screening revealed the presence of alkaloid which could be responsible for activity[13]. Antibacterial activity of *C. fenestratum* was found to be mainly due to the presence of berberine[14]. Antibacterial and Antifungal activity of *Curcuma aromatica* and *C. fenestratum* was investigated[3].

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