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Studies on antimicrobial activity of a critically endangered medicinal plant *Nardostachys jatamansi*

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ABSTRACT

Nardostachys jatamansi, a critically endangered rhizome-bearing medicinal plant, is restricted to specialized habitats in high altitudes of the Himalaya. The present study describes the antimicrobial activity of steam distillate of *N.jatamansi* against bacteria and fungi. The antibacterial activity was assessed in liquid media and poison food technique was employed to study antifungal efficacy. A significant reduction in the bacterial growth was observed suggesting the presence of antibacterial principles in the distillate fraction. Gram positive bacteria were found to be more inhibited than Gram negative bacteria. Marked antifungal activity was not observed but a good activity was observed against *A.terreus*. Since the study involved enteric bacteria and bacteria causing food poisoning, the distillate could be employed to control the infections caused by enteric bacteria as well as food poisoning bacteria. The results of the study validate the use of the plant in traditional medicine.

KEYWORDS

Nardostachys jatamansi;
Steam distillate;
Antimicrobial activity;
Optical density;
Poison food technique.

INTRODUCTION

Nardostachys (Family Valerianaceae) is a small herbaceous Himalayan genus, represented by two broad range endemic species, *N.grandiflora* and *N.jatamansi* in India. Red Data Book of Indian Plants includes *N.jatamansi* considered vulnerable at high altitudes. *N.jatamansi*, a critically endangered rhizome-bearing medicinal plant, is restricted to specialized habitats in high altitudes of the Himalaya, ranging from 3000 to 5000 m asl. The plant is collected from natural habitats for local consumption and trade. The underground part of *N.jatamansi* is used as a substitute for valerian and the extracts find use 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[1]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition[2]. Essential oils are products, generally, of rather complex composition comprising the volatile principles contained in the plants, and more or less modified during the preparation process[3]. Essential oils can be extracted using a variety of methods, although some are not commonly

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used today. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off[4]. Literature survey did not reveal much works being carried on the antimicrobial activity of *N.jatamansi*. The present study describes the potential of steam distillates, containing essential oil components, of *N.jatamansi* roots to inhibit Gram positive and Gram negative bacteria and species of *Aspergillus*.

MATERIALS AND METHODS

Collection of plant material and distillation

The dried rhizome of *N.jatamanis* was collected from the local herbal drug supplier in Udupi, Karnataka and mechanically powdered. A simple quick-fit apparatus with a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel, was used for the steam distillation. A known weight of (100 grams) air-dried material was subjected to steam distillation in the assembly. When heated up, the plant cells release their components and some of them are volatilized and carried by the steam. The volatile components were collected into the receiving flask during 3 hours of steam distillation[5,6]. The distillates were transferred into clean containers and stored in refrigerator until use.

Screening for antibacterial activity

The antibacterial activity of steam distillates was tested in liquid nutrient media[7] with minor modifications. The nutrient broth containing known volume of steam distillate of *N.jatamansi* was sterilized by autoclaving and inoculated with standardized volumes of 24 hours old broth cultures of test bacteria namely *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* followed by incubation at 37^0C for 24 hours. A set of nutrient broth tubes inoculated with bacterial cultures was kept as control without adding steam distillates. After incubation, the contents in the tubes were mixed and the optical density was measured by spectrophotometer at a wavelength of 490 nm as a guide to microbial growth and compared with control to assess antibacterial activity.

Screening for antifungal activity

In the study, species of the genus *Aspergillus* were selected as target fungi which are known to cause opportunistic mycotic infections in susceptible individuals. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[8]. The antifungal activity was assessed using Poison food technique[9]. The test fungi was allowed to grow in Sabouraud's dextrose agar plates poisoned with steam distillates (10% concentration). The effect of extract on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control plates which were not poisoned with the distillate. The experiment was done in triplicate and average reading was recorded.

RESULTS AND DISCUSSION

TABLE 1 shows antibacterial activity of steam distillate of *N.jatamansi* against Gram positive and Gram negative bacteria. A marked antibacterial potential of the distillate was observed in case of *B.subtilis* (40.60% inhibition) which is followed by *S.aureus* (40.00%), *E.coli* (38.40%), *K.pneumoniae* (36.60%) and *P.aeruginosa* (18.50%). Among bacteria tested, Gram positive bacteria were found to be more susceptible to steam distillate than Gram negative bacteria. *P.aeruginosa* was found to be least affected by the distillate. Two bacteria namely *E.coli* and *K.pneumoniae* tested were the members of Enterobacteriaceae and two bacteria namely *E.coli* and *S.aureus* were found to be involved in food poisoning.

TABLE 1 : Antibacterial activity of steam distillate of *N.jatamansi*

Distillate	Optical density at 490 nm				
	E.coli	*B.subtilis*	*S.aureus*	*K.pneumoniae*	*P.aeruginosa*
Control	0.606	0.208	0.688	0.525	0.489
N.jatamansi	0.373 (38.40)	0.123 (40.60)	0.412 (40.00)	0.332 (36.60)	0.398 (18.50)

Results are average of three trials (each trial includes two broth tubes), Values within parentheses- percentage inhibition as compared to control

TABLE 2 : Antifungal activity of steam distillate of *N.jatamansi*

Steam distillate	Average colony diameter in cm			
	A.niger	*A.oryzae*	*A.nidulans*	*A.terreus*
Control	4.3	3.8	3.7	2.6
N.jatamansi	3.9 (09.30)	3.5 (07.89)	3.4 (08.10)	1.6 (40.74)

Results are average of two trials (each trial includes duplicates), Values within parentheses- percentage inhibition as compared to control

Thus, the distillate could be used in the prevention and control of infections caused by enteric bacteria and food poisoning bacteria. *S.aureus* and *P.aeruginosa* are positively associated with hospital infections and most strains are often antibiotic resistant. Thus, the distillate tested here could be one of the approaches to control infections caused by these bacteria. The steam distillate of *N.jatamansi* was not found to possess marked antifungal activity against fungi tested (TABLE 2). The possible reason could be the low concentration of bioactive agents present in the distillate. The inhibition of test fungi was maximum in the case of *A.terreus* (4074% inhibition) followed by *A.niger* (09.30%), *A.nidulans* (08.10%) and *A.oryzae* (07.89%). Purified essential oil components of the rhizome could be more potent in inhibition of fungi tested.

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. The literature survey undertaken does not revealed much works on antimicrobial activity of the plant. Antimicrobial activity of ethanol, ethyl acetate and hexane extracts of *N.jatamansi* roots were studied *in vitro* against gram positive bacteria (*Stayphylococcus aureus, Streptococcus intermedius, S.faecalis, Bacillus pumilus, B.cereus, B.subtilus*), six gram negative bacteria (*Escherichia coli, Salmonella typhi, S.paratyphi B, Klebsiella pneumoniae, Proteus mirabilus, Shigella flexneri*) and five fungi (*Trichophyton rubrum, T.schoenleinii, Aspergillus niger, Candida albicans, C.glabrata*). 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].

The potential of *N.jatmansi* as a memory enhancer in mice was studied[12]. The 200 mg/kg dose of *N.jatmansi* ethanolic extract significantly improved learning and memory in young mice and also reversed the amnesia induced by diazepam (1 mg/kg, i.p.) and scopolamine (0.4 mg/kg, i.p.). Ethanol extract of the roots of *N. jatamansi* DC. was studied for its anticonvulsant activity and neurotoxicity in rats[13]. The results demonstrated a significant increase in the seizure threshold by *N.jatamansi* root extract against maximal electroshock seizure model as indicated by a decrease in the extension/flexion ratio. A 50% ethanolic extract of the rhizomes of *N. jatamansi* was shown to possess hepatoprotective activity[14]. Depressive activity of drug extract of Brahmi, Jatamansi and Shankhpushpi and their combined effects were studied on 66 mice of either sex. Lower dose (50 mg/kg) of all three drug extract showed comparatively less depressive effect while at higher doses each drug showed marked depressant activity. The order of potency was found to be Jatamansi > Shankhpushpi > Brahmi[15]. Hydroalcohol extracts of six herbs, including *N. jatamansi*, used in Indian systems of medicine, were tested for *in vitro* acetylcholinesterase inhibitory activity[16]. The results showed that the hydroalcohol extract from *N.jatamansi* inhibited 50% of AChE activity at concentrations of 100-150g/mL. The sumind, a polyherbal formulation containing *N. jatamansi* as one of the ingredient, exhibited significant antidepressant activity, as indicated by its ability to decrease swim stress induced immobility time in rats[17]. Ethanolic extract of *N.jatamansi* roots, an antioxidant and enhancer of biogenic amines, can slow the neuronal injury in a 6-OHDA-rat model of Parkinson's was evaluated and results of the study indicated that the extract of Jatamansi might be helpful in attenuating Parkinsonism[18]. *N.jatamansi* is restricted to some specialized habitats and is subject to destructive harvesting (removal of root/rhizome) from the wild. *Ex situ* conservation of the species assumes greater

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significance especially in a scenario when harvesting potential from the wild falls short of the demand for commercial exploitation[1].

CONCLUSION

N.jatamansi, a critically endangered rhizome-bearing medicinal plant, is having immense medicinal properties. The present paper validates another significance of the plant in terms of antimicrobial activity of the steam distillate against bacteria and fungi. The results of study reveal the potential of plant to inhibit bacteria and fungi *in vitro*. Further experiments in animal models could possibly reveal the potential of plant to inhibit disease causing microorganisms.

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