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Antibacterial and Antifungal Screening of the Root Extracts of *Nardostachys jatamansi*

Tehmina Sohail[a]*, Zahra Yaqeen[a], Hina Imran[a], Sohail Shaukat[b] and Atiq-ur-Rahman[a]

[a]Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi-75280, Pakistan

[b]Dr. Panjwani Centre for Molecular Medicine and Drug Research, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

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Abstract. Antimicrobial activity of ethanol, ethyl acetate and hexane extracts of *Nardostachys jatamansi* roots were studied *in vitro* against six pathogenic 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 (MIC) were between 0.5-1 mg/ml against all the studied microorganisms.

Keywords: *Nardostachys jatamansi*, antibacterial activity, antifungal activity, root extract

Introduction

Infectious diseases are the leading cause of global premature deaths, killing almost 50,000 people every day. In recent years, drug resistance of human pathogenic bacteria has been commonly reported from all over the world (Robin *et al.*, 1998; Davis, 1994; Mulligen *et al.*, 1993; Singh *et al.*, 1992; Piddock and Wise, 1989). The situation has become alarming in the developing as well as the developed countries due to indiscriminate use of antibiotics. The present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from alternative sources including plants.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995; Chopra *et al.*, 1992; Iyengar, 1981). The substances that can either inhibit the growth of pathogens or kill them and have nil or least toxicity to host cells are considered candidate for developing new antimicrobial drugs. In the present study, the root of *Nardostachys jatamansi* was selected for screening against some pathogenic bacteria and fungi, based on its traditional use.

Nardostachys jatamansi, commonly called "Ferula", "sumbal" or "musk" root of commerce, belongs to family Valerianaceae and is grown in Indian Himalayas, Nepal, Bhutan and Pakistan. (Nadkarni, 1954). A conclusive data is available on the therapeutic potential of the plant which reveals that the infusion prepared from fresh roots is employed in the treatment of spasmodic hysterical affections, especially the palpitation of heart, nervous disorder, headache, chorea, flatulence etc. Volatile oils extracted from the rhizome of *N. jatamansi* are used in many diseases of digestive and respiratory organs and in jaundice. Their use has been recommended in typhus fever, gastric spasm, hysteria, delirium tremens, diarrhoea, dysentery, leucorrhoea, gleets, chlorosis, asthma, chronic bronchitis and other maladies accompanied with asthmatic condition (Narayan and Kumar, 2003; Chopra *et al.*, 1958; Nadkarni, 1954).

N. jatamansi and *N.chinensis* have been used in the preparation of healthy food from chitin and of traditional Chinese medicines for skin care, in the treatment of acne and diseases of stomach and spleen (Qiong, 2005; Xing, 2005; Yang *et al.*, 2005).

An ample data regarding the phytochemical analysis of the plant is available. The rhizome volatiles contained 72 components, of which 41 constituting 70% of the oil, were positively identified. The oil is composed of nine monoterpenes (1.7%), 25 sesquiterpenes (43.9%) and 7 non-terpenic components (24.4%). The predominant sesquiterpenes were, nardol (10.1%), α-selinene (9.2%), β-caryophyllene (3.3%), cubebol (2.9%), α-gurjunene (2.5%), γ-gurjunene (2.3%) and α-humulene (2.3%) (Mahalwal and Mohammad, 2002).

Present work is an attempt towards screening of antimicrobial agents of plant origin (Clark, 1996), which can have numerous

*Author for correspondence; E-mail: sohailshaukat786@yahoo.com

potential therapeutic effects relating to the treatment of diseases and infections; besides, they can also mitigate many side effects, often associated with synthetic drugs, which in turn will reduce the burden of multiple drug resistance.

Materials and Methods

Collection and identification of plant material. Plant material was purchased from the local market and properly identified by a taxonomist.

Preparation of plant extracts. Plant extract was prepared by the method of Alad and Irobi (1993) with minor modifications. Briefly, 100 g of powdered plant material was soaked separately in 100 ml solvent (each) of 70% ethanol, ethyl acetate and hexane for 72 h, at the room temperature. The mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper # 1. The filtrates obtained were concentrated *in vacuo* at 30 °C and stored at 4 °C until further use.

Preparation of samples. All the extracts were dissolved in 6% dimethylformamide (DMF), yielding the strength of 20 mg/ml (2%) from which further dilutions were made in the same solvent. Ampicillin and Nystatin were used as reference standard (positive control) in the same strength/concentration, while 6% dimethylformamide (DMF) was used as negative control.

Microorganisms used. The test organisms used included six gram positive bacteria (*Staphylococcus aureus, Streptococcus faecalis, S. intermedius, Bacillus subtilis, B. pumilus, B. cereus*), six gram negative bacteria (*Salmonella typhi, S. paratyphi B., Eschrichia coli, Klebsiella pneumoniae, Proteus mirabilus* and *Shigella flexneri*) and five fungi (*Aspergillus niger, Trichophyton rubrum, T. schoenleinii, Candida albicans* and *C. glabrata*). All the test organisms used in the present study were acquired from the Department of Microbiology, University of Karachi. Before assessing the antimicrobial activity, purity of cultures was fully ascertained by standard method of characterization.

Culture media and inocula. Tryptic soya agar (Merck) for gram positive and gram negative microorganisms and Sabouraud dextrose agar (Merck) for fungi were used. Microbial cultures (bacteria grown at 37 °C and fungi at 25 °C) were appropriately diluted in sterile normal saline water to obtain the cell suspension at 10^6 CFU/ml.

Antibacterial activity. Agar well diffusion method (Ahmad *et al.*, 1998) was used for assessing the antibacterial activity of the test material. According to this method, 0.1 ml of the diluted inocula were thoroughly mixed with 20 ml of molten sterile soya tryptic agar and poured into sterilized petri dishes. All plates were left to set at 4 °C for 30-40 min. Holes of 6 mm diameter were made in the centre of each seeded plate which were then filled aseptically with 0.1 ml of test solutions and marked accordingly. 6% DMF solution was used as negative control. All the plates were then incubated at 37 °C ± 1 °C for 24 h and the zones of inhibition exhibited by different extracts in various concentrations were measured. All plates were run in triplicate.

Antifungal activity. The antifungal activity of the test material was determined by agar tube dilution method (Paxton, 1991). Test tubes having sterile Sabouraud dextrose agar were inoculated with test solutions of different concentrations and kept in slanting position at the room temperature for solidification. The test fungal cultures were inoculated on the slants, incubated at 25 °C for 7 days and growth inhibition was observed after the 7th days (Washington and Sutter, 1980). Nystatin, used as the standard antifungal drug, is a polyene antifungal antibiotic, derived through fermentation by *Streptomyces*. It is one of the drugs used to combat fungal infections and has been in use for the treatment of cutaneous, vaginal, and oral fungal infections since 1950s (Physicians Desk Reference, 1998; Stark, 1967; Pace and Schantz, 1956).

MIC determination. The MIC values of ethanolic extract of *N. jatamansi* roots were determined against the gram positive and gram negative bacteria, and fungi (10^6 CFU/ml) by the serial dilution technique (Reiner, 1982).

Results and Discussion

In the present study, roots of *N. jatamansi* were extracted in three different solvents namely, ethanol, ethyl acetate and hexane. These extracts were tested against some pathogenic bacteria and fungi. The antimicrobial activity of different extracts and their relative potencies were quanti-tatively assessed by the presence or absence of inhibition zones and their diameter as given in Table 1 and 2, respectively. Antifungal activity is given in Table 3, whereas, Table 4 and 5 represent minimum inhibitory concentration (MIC) of ethanolic extract.

The study not only gives preliminary account of the antimicrobial substances in the root of *N. jatamansi* but also points out the active solvent which holds the maximum activity. From the study, it is evident that a considerable difference in the activity exists among various solvents used for extraction. The ethanol was the best among the solvents used for extraction. In the present study, ethanolic extract exhibited maximum antimicrobial activity which was considered as 100% in relative terms. Ethyl acetate and hexane extract, exhibited relatively less activity against gram positive,

Table 1. Antibacterial activity exhibited by roots of *Nardostachys jatamansi* against gram positive organisms

Name of organisms	Zone of inhibition; dia. (mm)												Negative control (DMF)
	Ethanolic extract (mg/ml)			Ethyl acetate extract (mg/ml)			Hexane extract (mg/ml)			Ampicillin (mg/ml)			
	5	10	20	5	10	20	5	10	20	5	10	20	
Staphylococus aureus	C	B	A-I	D	C	B	-	-	C	C	B	B	-
Streptococcus intermedius	B	B	A	C	C	B	D	D	C	C	C	B	-
Streptococcus faecalis	C	C	B	-	-	-	-	-	-	-	-	-	-
Bacillus pumilus	C	C	B	D	D	C	-	-	-	C	B	A	-
Bacillus cereus	B	B	A	D	C	B	C	D	D	C	B	B	-
Bacillus subtilus	C	B	B	D	C	C	-	-	-	-	-	-	-

Categorization of inhibition zone: A-1=35-39 mm; A=30-34 mm; B=25-29 mm; C=20-24 mm; D=15-19 mm

Table 2. Antibacterial activity exhibited by roots of *Nardostachys jatamansi* against gram negative organisms

Name of organisms	Zone of inhibition; dia. (mm)												Negative control (DMF)
	Ethanolic extract (mg/ml)			Ethyl acetate extract (mg/ml)			Hexane extract (mg/ml)			Ampicillin (mg/ml)			
	5	10	20	5	10	20	5	10	20	5	10	20	
Escherichia coli	C	B	A	D	B	B	D	C	B	C	B	A	-
Salmonella typhi	C	C	B	-	C	B	-	-	-	C	C	B	-
Salmonella paratyphi B	C	B	A	D	C	B	-	-	-	-	-	-	-
Klebsiella pneumoniae	C	B	A	D	C	B	D	D	C	D	D	D	-
Proteus mirabilus	B	B	B	-	-	-	-	-	-	-	-	-	-
Shigella flexneri	C	B	A	D	C	B	-	-	B	C	C	B	-

Categorization of inhibition zone: A=30-34 mm; B=25-29 mm; C=20-24 mm; D=15-19 mm

gram negative bacteria and fungi as compared to the standard and the ethanolic extract as shown in Table 1-3. Hexane and the ethyl acetate extracts, both exhibited less antifungal activity as compared to the standard (Nystatin) and ethanolic extract. It has also been reported that only alcoholic extract was the better solvent for extraction of antimicrobially active substances as compared to water and hexane (Ahmad *et al.*, 1998). Difference existing in total percentage of activity displayed by test organisms and reference standard might be due to indiscriminate use of antibiotics which may have reduced the sensitivity of standard antibiotics.

Among the gram positive bacteria, ethanolic extract showed much better activity against *S. aureus* and *B. subtilus*. Both the organisms exhibited A-1 (excellent) category zone of inhibition. Other extracts and the standard Ampicillin failed to exhibit A-1 category zone. The antimicrobial activity against gram negative microorganisms was also comparable with the extracts in different solvents and with the standard. The results showed that at 20 mg/ml concentration, only *E. coli* exhibited A-1 zone of inhibition in the ethanolic extract as compared to the other solvent extracts and the standard. Similarly ethanolic extract exhibited better antifungal and anticandidal activity as compared to other solvent extracts. *T. rubrum, C. albicans, C. glabrata*, exhibited A-1 category of inhibition activity at 20 mg/ml concentration. Antifungal activity of the root extract of *N. jatamansi* has been verified by the work of Bindra *et al.* (2001) who reported that antifungal herbal formulation containing the root extract of *N. jatamansi*, has been used for the treatment of human nail infection. This clearly indicates that antibiotic resistance does not interfere with the antimicrobial action of plant extract but this extract might have different modes of action on test organisms.

Antimicrobial activity of the essential oil of the roots of *N. jatamansi* was also assessed previously against other strains of bacteria and fungi (Sridhar *et al.*, 2003; Rao *et al.*, 1986); it showed good antimicrobial activity with varying degrees of potency. The difference in potency may be due to the stage of collection of plant sample, the difference in the activity in essential oil, different solvents, and different sensitivity of the test strains and method of extraction (Nimri *et al.*, 1999).

Table 3. Antifungal activity exhibited by roots of *Nardostachys jatamansi* against yeast and fungi

Name of organisms	Zone of inhibition; dia. (mm)												
	Ethanolic extract (mg/ml)			Ethyl acetate extract (mg/ml)			Hexane extract (mg/ml)			Nystatin			Negative control
	5	10	20	5	10	20	5	10	20	5	10	20	(DMF)
Trichophyton rubrum	B	A	A-1	B	A	A	B	A	A	B	A	A	-
Trichophyton schoenleinii	B	A	A-1	B	B	A	B	B	A	B	A	A	-
Aspergillus niger	-	C	B	-	-	-	-	-	-	C	B	A	-
Candida albicans	A	A	A-1	B	B	A	C	B	A	B	A	A	-
Candida glabrata	A	A	A-1	B	B	A	C	B	B	B	A	A	-

Categorization of inhibition activity: A-1 = excellent activity; A = good activity; B = moderate activity; C = low activity

Table 4. Minimum inhibitory concentration of ethanolic extract against gram negative and gram positive bacteria

Name of organisms	Concentration (mg/ml)							
	20	10	5	2.5	2	1	0.5	0.25
Staphylococcus aureus	-	-	-	-	-	-	+	+
Streptococcus intermedius	-	-	-	-	-	-	-	+
Streptococcus faecalis	-	-	-	-	-	-	+	+
Bacillus pumilus	-	-	-	-	-	-	+	+
Bacillus subtilus	-	-	-	-	-	-	+	+
Bacillus cereus	-	-	-	-	-	-	+	+
Escherichia coli	-	-	-	-	-	-	-	+
Salmonella typhi	-	-	-	-	-	-	-	+
Salmonella paratyphi B	-	-	-	-	-	-	-	+
Klebsiella pneumoniae	-	-	-	-	-	-	-	+
Proteus mirabilus	-	-	-	-	-	-	-	+
Shigella flexneri	-	-	-	-	-	-	-	+

Table 5. Minimum inhibitory concentration of ethanolic extract against yeast and fungi

Name of organisms	Concentration (mg/ml)							
	20	10	5	2.5	2	1	0.5	0.25
Trichophyton rubrum	-	-	-	-	-	-	-	+
Trichophyton schoenleinii	-	-	-	-	-	-	-	+
Aspergillus niger	-	-	+	+	+	+	+	+
Candida albicans	-	-	-	-	-	-	-	+
Candida glabrata	-	-	-	-	-	-	-	+

An attempt, therefore, has been made to determine the minimum inhibitory concentration of ethanol extract against gram positive and gram negative bacteria and fungi as shown in Table 4 and 5. MIC of the extract against the gram positive bacteria (*Staphylococcus aureus, S. faecalis, B. pumilus, B. subitlus,* and *B. cereus*), was found to be 1 mg/ml, whereas, that against the gram negative bacteria (*S. typhi, S. paratyphi B., E. coli, K. pneumoniae, P. mirabilus* and *S. flexneri*), was 0.5 mg/ml. The value against the fungi was 0.5 mg/ml except *A. niger*, which is somewhat resistant and its MIC value was 5 mg/ml.

Roots of *N. jatamansi,* which have already shown the antimicrobial activity consist of multiple phytochemicals/ phytoconstituents, which have stimulating effect (Singh and Ali, 2003; Mahalwal and Mohammad, 2002). In the present study, these multiple components have been further sub-divided according to their solubility in different solvents and categorized relatively against a number of pathogenic microorganisms. It can be concluded safely that ethanolic fraction of *N. jatamansi* roots, have the most active components and can be a good source of the chemical compounds of pharmaceutical importance and that the fraction has great potential to be the source of future broad spectrum antimicrobial compound. Further studies are in progress.

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