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Research Article

Antimicrobial activities of *Nardostachys jatamansi* extract against multidrug resistant bacterial species

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Abstract

At present, multidrug resistant (MDR) bacteria have become widespread worldwide, leading to high morbidity and mortality rates in bacterial infections. Again, as there is practically no new antimicrobial agent in the pipeline, this will create a threat to humanity for their survival. In this study, we explored the possible antimicrobial action of ethanolic extract of a typical plant of West Bengal, *Nardostachys jatamansi*, against MDR and American Type Culture Collection (ATCC) strain bacteria. Antimicrobial activities of *Nardostachys jatamansi* ethanol extract were studied by disc diffusion technique, and then minimum inhibitory concentration (MIC) determination was done by serial dilution in Mueller Hinton broth. Ethanolic extract of *Nardostachys jatamansi* showed antimicrobial activities with MIC varied between 2.77- 5.82 mg/mL in both MDR and ATCC bacteria. Ethanolic extract of *Nardostachys jatamansi* is an effective antimicrobial agent on MDR bacteria and may help save the lives of many critically ill patients.

Keywords: *Nardostachys jatamansi*, MDR bacteria, antimicrobial action, MIC value

Introduction

Plants have been used as the source of medicines for the ages to treat various diseases. Irrespective of the availability of various synthetic drugs, plants are being used as an integral part of health care in different countries. Many medicinal plants are commonly reported as endangered and have a high risk of extinction by the natural environment or by humans. As *Nardostachys jatamansi* (Family Caprifoliaceae) is one of the endangered plants, all measures must be taken to protect it [1].

The *Nardostachys* plant is native to the Himalayas and is a member of a genus of other tiny herbaceous plants. The two most prominent families of endemic plants in India are called *Nardostachys jatamansi* and *Nardostachys grandiflora*, respectively. The *Nardostachys jatamansi* plant is listed in the Red Data Book because it is thought to be vulnerable at high altitudes [2]. At elevations ranging from 3,300 to 5,000 meters, the medicinal plants can be found across India's Himalayan range. In most cases, it will be discovered to be growing on an open, stoney slope that also has grass on it. In addition to these locations, Sikkim, South-West China, Afghanistan, Nepal, and Pakistan are also home to its population [3-5].

Multiple drug-resistant infections are significant global public health problems [6]. After combining the several data with evidence, it has been shown that 30% of prescriptions of antibiotics are inappropriate [7], and antibiotic-resistant bacteria will persist in being a significant threat in the near future. A recent report by the US CDC (Centers for Disease Control and Prevention) on the burden and trends of main antibiotic-resistant bacteria, including some usually associated with healthcare, has been published [8,9]. Recently, scientific interest in medicinal plants has increased, especially concerning antimicrobial action. The global revival of curiosity in herbal drugs, unwanted side effects, the mounting cost of conventional remedies, prolonged side effects, and scarcity of effective synthetic medicines have left scientists to find other alternatives, particularly herbal ones. Essential oils of *Nardostachys jatamansi* have shown a broad range of activity, including action against diverse viruses, bacteria, and fungi [10].

Nardostachys jatamansi's underground section is used in around twenty-six Ayurvedic medicines. Hypertension, heart disease, and sleeplessness are all treated using the root. The root and rhizome's bioactive compounds include sedative, tranquillizing, antispasmodic, and carminative properties [11]. The plant's roots and ribosomes are rich in sesquiterpenes (jatamansic acid, valerone, jatamansol, dihydrojatamansin, jatamansone, nardosatchone) and coumarins, which produce a yellow-colored essential oil with a pleasant odour. Jatamol A, jatamol, spirojatamol, jatamansinone, nardosinone, oreoseoloi, oreselone, oroseolol, valeranal, seychelane, sugar, starch, and others are some of the other ingredients [12].

In traditional medicine, the roots and rhizomes of *Nardostachys jatamansi* are used as an anti-stress remedy, and in India, it is available as an Ayurvedic anticonvulsant drug, Ayush 56 [13]. The aroma of the rhizome is being used as an add-on agent in medicinal oils and supporting hair growth and its natural black colour [14]. The essential oil of the roots of this plant showed toxic action on fungus [15], antimicrobial [16], antifungal [17], antihypertensive [18], cardiac anti-arrhythmic [19], and anticonvulsant activities [19,20]. The ethanol extract (50%) of the rhizome demonstrated hepatoprotective [21], antihyperlipidemic [22], and cardiac antiarrhythmic activities [23]. In traditional and other systems of medicine, the roots of the plant are also used to treat indurations and solid tumours [24,25]. It has been reported by Bhagat et al. the cytotoxicity of n-butanol fraction and alcoholic extract of *Nardostachys jatamansi* in cancer cell lines of liver, lung, prostate and ovary. Besides this, cytotoxicity of two new sesquiterpenoids and the crude extract by chloroform: methanol, have been studied in ER-positive breast cancer, lung, prostate, and neuroblastoma cell lines [25,26]. *Nardostachys jatamansi* and *Nardostachys chinensis* have been used in chitin-based foods, skin care, traditional Chinese medicines for acne, and gastric and splenic diseases [27].

The present study was undertaken to assess the antibacterial agents of the plant *Nardostachys jatamansi*, which can have various possible therapeutic effects relating to the treatment of bacterial infectious diseases; besides, they can also alleviate many adverse drug effects, frequently linked with synthetic drugs, which in turn will diminish the prevalence of multiple drug resistance. Thus, the present study aimed to assess the antimicrobial properties of the 70% ethanolic root extract of *Nardostachys jatamansi* plant on MDR bacteria and ATCC control strains.

Materials and Methods

Collection and identification of plant material

Plant material (Figure 1) was procured from the standard Ayurvedic shop expertise over 150 years (Adi Ambika Ausadhalaya, 2B, Shyama Prasad Mukharjee Rd, Jatin Das Park, Patuapara, Bhowanipore, Kolkata, West Bengal), an expert there identified the plant as *Nardostachys jatamansi* from where the roots were collected. Later the identification was authenticated by Dr. Tapan Seal, Scientist "D", Botanical Survey of India, Ministry of Environment, Forest & Climate change, Government of India (Ref. no.: BSI/PLANT CHEM/0011-2022; Sample ID: WBSU/SD/Microbiol/001) as *Nardostachys jatamansi* **(D. Don) DC** (Family: Caprifoliaceae).

Figure 1. Root of *Nardostachys jatamansi*.

Preparation of plant extracts

The dried root part of the plant was powdered and extracted with 70% ethanol. One gram of powdered root material was impregnated in 2 mL 70% ethanol solvent (~500 mg/mL). The extract was thoroughly mixed by vortex for 20 min and incubated at room temperature for 24 h (Figure 2). After 24 h, it was again mixed by vortex for 15-20 min and then centrifuged (3000 rpm) for 10 min. After centrifugation, about 1 mL of supernatant (Figure 3) was collected and used for the subsequent procedures or stored at 4°C until further use.

Tested microorganisms

The strains of *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR strain, Table 1), *Klebsiella pneumoniae* (MDR strain, Table 2), and *Pseudomonas aeruginosa* (MDR strain, Table 3) were used in this experiment. Their antimicrobial sensitivities are shown in Tables 1-3. These tested organisms were acquired from the Department of Microbiology, Peerless Hospital, Kolkata, West Bengal, India. Before studying the antibacterial activity, standard methods or characterisations determined the purity of cultures.

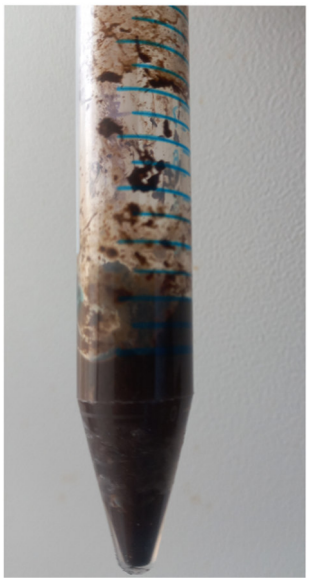


Figure 2. Plant material after vortex.

Culture media and inoculum

Mueller Hinton (MH) agar media was used for the disc diffusion method, and MH broth was used for MIC determination. Bacterial cultures were diluted correctly with sterile normal saline to obtain the cell suspension of 0.5 McF (McFarland) by measuring in the DensiCHEK.

Disc preparation

Filter paper discs were kept in the prepared ethanolic plant extract for 10-15 min and dried in laminar airflow. Simultaneously discs were prepared for control with only 70% ethanol for the same period and then dried.

Antibacterial activity

The agar disc diffusion method carried out the antibacterial activity, followed by a microdilution test for minimum inhibitory concentration (MIC) determination.

Table 1. Antibiotics sensitivity of tested *Escherichia coli* (MDR) in VITEK 2 automated system.

Selected Organism: Escherichia coli **Source:** URINE					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Amoxicillin/Clavulanic Acid	>=32	R	Meropenem	>=16	R
Piperacillin/Tazobactam	>=128	R	Amikacin	4	S
Cefuroxime	>=64	R	Gentamicin	<=1	S
Cefuroxime Axetil	>=64	R	Ciprofloxacin	>=4	R
+Cefixime	Extrapolated	R	+Levofloxacin	Extrapolated	R
Ceftriaxone	>=64	R	Tigecycline	<=0.5	S
Cefoperazone/Sulbactam	>=64	R	Fosfomycin	<=16	S
Cefepime	>=32	R	Colistin	<=0.5	I
+Doripenem	Extrapolated	R	+Polymixin B	Extrapolated	I
Ertapenem	>=8	R	Trimethoprim/	>=320	R
Imipenem	>=16	R			

R= Resistant, S= Susceptible, I= Intermediate

Disc diffusion method

In order to evaluate the effectiveness of the test substance as an antibacterial, the agar disc diffusion technique was utilized. In this procedure, uniform suspensions of four different strains of bacteria were made, each containing (0.5 McF), and were then mixed appropriately. Afterwards, they were spread uniformly on separate sterile petri dishes containing solid MH agar. Additionally, the control disc that contained 70% ethanol was utilized. The placement of the discs in the petri dish was meticulously managed to ensure that they were kept apart from one another and did not get extremely close to the petri dish's edge. After incubating all of the plates overnight at 37°C, the zones of inhibition that were demonstrated by the various strains of bacteria were subsequently measured.

Micro-dilution test

MIC determination

The 96- well sterile microtiter plate was used to determine the MIC. 100 mL of MH broth was pipetted in each of the wells. The 100 ml extract was then added to the first well and thoroughly mixed, then serially diluted in successive wells in double dilutions. Then 10 mL of 0.5 McF bacterial suspensions were pipetted in each well, and the control experiment was also done similarly using only 70% ethanol. The optical density readings were analysed with a Thermo MULTISKAN EX microplate reader. MIC values were detected at 620nm at zero h and 24 h. During incubation, plates were incubated under normal atmospheric conditions at 37°C for 24 h. The MIC value was determined as the lowest concentration of the root extract in the MH broth medium that inhibits the growth of the test microorganism.

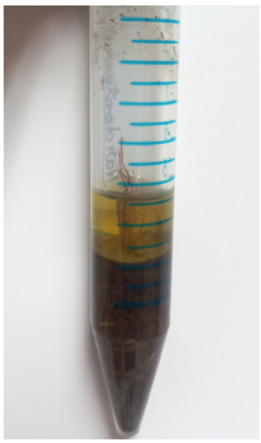


Figure 3. Separated supernatant after certification.

Results

Agar disc diffusion and MIC methods were used to assess the antibacterial activity of ethanolic root extracts of *Nardostachys jatamansi* against different MDR strains of bacterial species. The antibacterial activity of extract and their relative efficiency were evaluated by observing the inhibition zones and their diameter. In the present study, the result of the disc diffusion method showed that the extract inhibited the growth of all tested microorganisms, as shown in Table 4. It has been shown that the extract acts best against Pseudomonas aeruginosa (MDR) because among all plates, only P. aeruginosa (MDR) plate showed a maximum zone of inhibition around the discs (about 8 mm in diameter). In the case of E. coli (ATCC), E. coli (MDR) and K. pneumoniae (MDR) plates showed a slight zone of inhibition around the discs.

Table 2. Antibiotics sensitivity of tested *Klebsiella pneumoniae* (MDR) in VITEK 2 automated system.

Selected Organism: Klebsiella pneumoniae **Source:** WOUND SWAB					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Amoxicillin/Clavulanic Acid	>=32	R	Meropenem	8	R
Piperacillin/Tazobactam	>=128	R	Amikacin	32	I
Cefuroxime	>=64	R	Gentamicin	>=16	R
Cefuroxime Axetil	>=64	R	Ciprofloxacin	>=4	R
+Cefixime	Extrapolated	R	+Levofloxacin	Extrapolated	R
Ceftriaxone	>=64	R	Tigecycline	1	S
Cefoperazone/Sulbactam	32	I	Fosfomycin	128	**R
Cefepime	>=32	R	Colistin	>=16	**I
+Doripenem	Extrapolated	R	+Polymixin B	Extrapolated	**I
Ertapenem	>=8	R	Trimethoprim/ Sulfamethoxazole	>=320	R
Imipenem	>=16	R			

*= AES (Advanced expert system) modified, **= User modified

MIC values show an exact pattern of growth inhibition against all tested microorganisms. The root extract showed antibacterial activity against all the tested bacteria at concentrations of 5.82 mg/mL with a range of 2.77- 5.82 mg/mL. MIC of the extract against *E. coli* (MDR) was found to be around 12.23 mg/mL, whereas that against the *E. coli* (ATCC), *K. pneumoniae* (MDR) and *P. aeruginosa* (MDR) were within 2.77 - 5.82 mg/mL. The following graphs of differences between the absorbance values in the zero h and the 24 h versus the concentration of dilution of the plant extract demonstrate a similar pattern of sensitivity against MDR strains (Figures 4-7).

Discussion

From this experiment, we have established that *Nardostachys jatamansi* root extract has antimicrobial activities against different pathogenic MDR strains like *E. coli, K. pneumoniae* and *P. aeruginosa*. Many

studies have worked on the antimicrobial activities of *Nardostachys jatamansi* against different microorganisms, but no study reflects on the effectiveness of this plant extract over the MDR strains.

Table 3. Antibiotics sensitivity of tested *Pseudomonas aeruginosa* (MDR) in VITEK 2 automated system.

Selected Organism: Pseudomonas aeruginosa (Multi-drug resistant organism, MDRO) **Source:** URINE					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Amoxicillin/Clavulanic Acid			Meropenem	>=16	R
Piperacillin/Tazobactam	>=128	R	Amikacin	>=64	R
Cefuroxime			Gentamicin	>=16	R
Cefuroxime Axetil			Ciprofloxacin	>=4	R
+Cefixime			+Levofloxacin	Extrapolated	R
Ceftriaxone			Tigecycline	>=8	R
Cefoperazone/Sulbactam	>=64	R	Fosfomycin	128	I
Cefepime	>=32	R	Colistin	<=0.5	I
+Doripenem			+Polymixin B	n.a.	I
Ertapenem			Trimethoprim/ Sulfamethoxazole		
Imipenem	>=16	R			

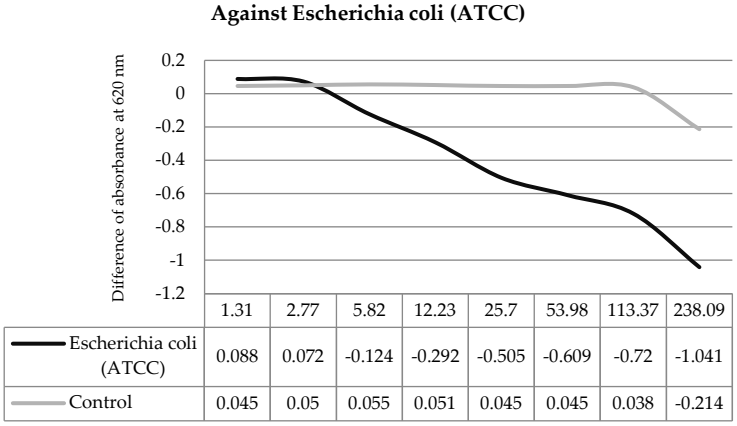


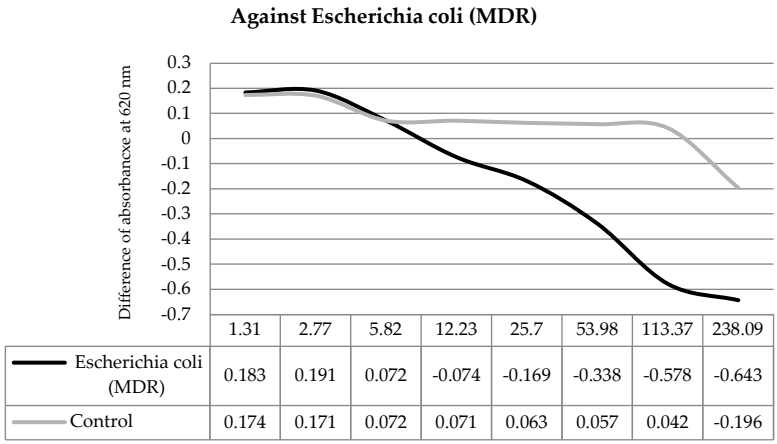
Figure 4. The antimicrobial activity of root extract Nardostachys jatamansi against Escherichia coli (ATCC). Concentrations of the extract are given in "X" axis.

This study showed that ethanolic root extract of *Nardostachys jatamansi* inhibited the growth of all the tested bacterial species. In one study, the MIC values of the plant extract against *E. coli* were 11.6 µg/mL, *K. pneumoniae* 21.4µg/mL and *P. aeruginosa* 14.5µg/mL [28]. In another study, the MIC value of the extract against the gram-negative bacteria (*S. typhi, S. paratyphi B., E. coli, P. mirabilis, K. pneumoniae* and *S. flexneri*) was 0.5 mg/mL [27].

Table 4. Antibacterial activity showed by root extract of *Nardostachys jatamansi* against tested microorganisms.

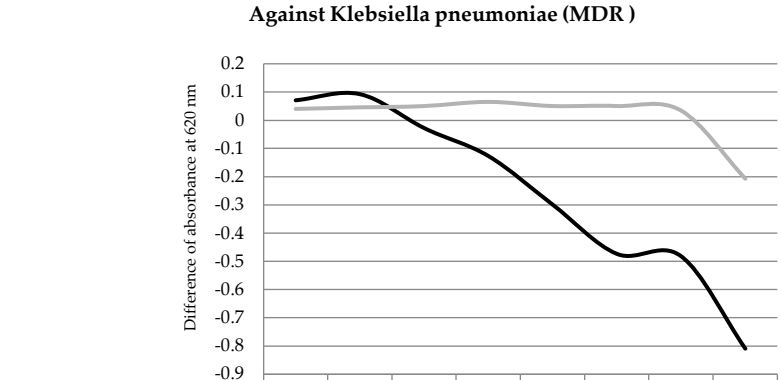
Name of organisms	Zone of Inhibition; diameter (mm)		
	Control (70% ethanol)	Plant extract	
		Disc I	Disc II
Escherichia coli (ATCC)	6	7	7
Escherichia coli (MDR)	6	7	6.5
Klebsiella pneumoniae (MDR)	6	6.5	7
Pseudomonas aeruginosa (MDR)	6	7	8

However, in this present study, we have noticed that the MIC value is around 5.82 mg/mL for the strains of *E. coli* (ATCC), *K. pneumoniae* (MDR) and *P. aeruginosa* (MDR) and 12.23 mg/mL for *E. coli* (MDR). In a previous study, it was indicated that the antimicrobial action of the plant extract does not interfere with antibiotic resistance, but it shows different modes of action on various microorganisms [29,30].



	1.31	2.77	5.82	12.23	25.7	53.98	113.37	238.09
Escherichia coli (MDR)	0.183	0.191	0.072	-0.074	-0.169	-0.338	-0.578	-0.643
Control	0.174	0.171	0.072	0.071	0.063	0.057	0.042	-0.196

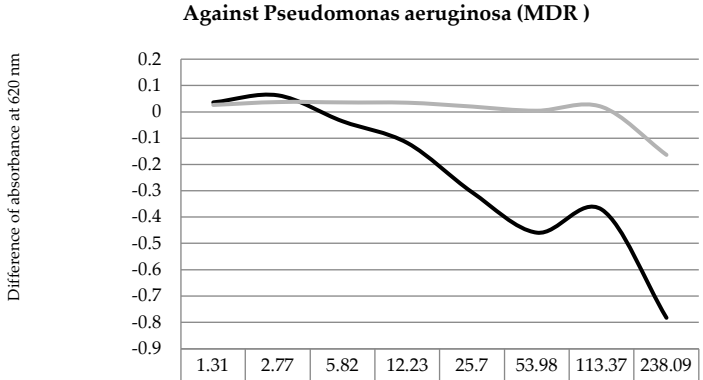
Figure 5. The antimicrobial activity of root extract Nardostachys jatamansi against Escherichia coli (MDR). Concentrations of the extract are given in "X"axis.



	1.31	2.77	5.82	12.23	25.7	53.98	113.37	238.09
Klebsiella pneumoniae (MDR)	0.071	0.093	-0.028	-0.126	-0.299	-0.473	-0.481	-0.809
Control	0.041	0.046	0.051	0.065	0.051	0.051	0.035	-0.207

Figure 6. The antimicrobial activity of root extract *Nardostachys jatamansi* against *Klebsiella pneumoniae* (MDR). Concentrations of the extract are given in "X" axis.

This study is the first report on the antimicrobial activity against MDR strains of different bacterial species of the ethanolic root extract of *Nardostachys jatamansi,* which appears to be due to different phytochemicals present in it [31,32].



	1.31	2.77	5.82	12.23	25.7	53.98	113.37	238.09
Pseudomonas aeruginosa (MDR)	0.036	0.063	-0.036	-0.118	-0.305	-0.459	-0.371	-0.782
Control	0.027	0.038	0.036	0.035	0.02	0.005	0.019	-0.163

Figure 7. The antimicrobial activity of root extract *Nardostachys jatamansi* against *Pseudomonas aeruginosa* (MDR). Concentrations of the extract are given in "X" axis.

Conclusion

The present study established that ethanolic extract of *Nardostachys jatamansi* roots has antimicrobial activities against MDR strains of tested bacterial species. The extract consists of various active components responsible for this antimicrobial activity. Thus, in future, this may be used to tackle MDR bacteria. However, further studies regarding this are required.

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Authors contribution

All the authors have contributed equally.

Conflict of interest

The authors declare no conflict of interest.

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