Shobha et al | Article 10516
J Pure Appl Microbiol. 2025;19(4):2923-2930. doi: 10.22207/JPAM.19.4.31
Received: 24 April 2025 | Accepted: 14 October 2025
Published Online: 27 November 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Potential of Aqueous and Ethanolic Extracts of Fruits of *Embelia ribes*, Rhizomes of *Nardostachys jatamansi*, and Stolons of *Picrorhiza kurroa*: An *In vitro* Investigation

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Abstract

The traditional system of medicines using herbs is highly practical even today both in urban and rural parts of India, where they are commonly used as dietary supplements as well as therapeutic agents. There is a need for herbal supplements that work effectively against microbes, including multidrug-resistant organisms. Hence, the current research aimed to identify the antibacterial and antifungal properties of aqueous and ethanolic extracts prepared from the fruit of *Embelia ribes*, the rhizome of *Nardostachys jatamansi* and the stolon of *Picrorhiza kurroa.* Both aqueous and ethanolic extracts of *Embelia ribes* fruit, *Picrorhiza kurroa* stolons and *Nardostachys jatamansi* rhizomes strongly inhibited the *Staphylococcus aureus* (ATCC 25923) strain. *Picrorhiza kurroa* stolon and *Nardostachys jatamansi* rhizome extracts have shown a maximum zone of inhibition for *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 25922) strains. *Nardostachy jatamansi* rhizome extracts are effective against all three bacterial strains mentioned above. None of the three herbs inhibited the *Candida albicans* (ATCC 90028) strain. The results revealed the antibacterial properties of the aqueous and ethanolic plant extracts. This study substantiates the use of these herbs in ayurvedic formulations that help in treating infectious diseases. Continued research into its antimicrobial properties and mechanisms will be essential for fully realizing its therapeutic potential.

Keywords: *Embelia ribes*, *Nardostachys jatamansi*, *Picrorhiza kurroa,* Antibacterial, Antifungal, Agar Well Diffusion, Zone of Inhibition

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Citation: Shobha KL, Almeida PMD, Pai KSR. Antimicrobial Potential of Aqueous and Ethanolic Extracts of Fruits of *Embelia ribes, Rhizomes* of *Nardostachys jatamansi*, and Stolons of *Picrorhiza kurroa*: An *In vitro* Investigation. *J Pure Appl Microbiol.* 2025;19(4):2923-2930. doi: 10.22207/JPAM.19.4.31

INTRODUCTION

The Indian system of medicine, Ayurvedic literature, compiles thousands of formulations prepared from natural resources, especially medicinal plants.[1] Traditional system medicines, which use herbs, are highly practiced even today both in urban and rural parts of India, in the form of regular dietary supplements as well as therapeutic agents,[2] as they have medicinal properties, easy availability, and cost-effectiveness, and people believe that they have fewer side effects. Studies have shown that different types of active phytochemicals are responsible for the therapeutic benefits of medicinal plants. They have antioxidant properties, nutritive, immune boosting, nootropic benefits and antibacterial effects.[3,4] Therefore, various formulations prepared from plant-based medicines are used for chronic health conditions such as skin infections, chronic lung infections, wound healing, diabetes, and tuberculosis.[5] In recent years, most organisms have developed resistance to standard antibiotic therapies; hence, higher levels of antibiotics, which are not only expensive but also have more side effects and create an economic burden for lower middle-class people, are needed.[6] These findings emphasize the need for complementary alternative therapeutic agents that prevent infections, improve the immune system, effectively work against multidrug-resistant organisms that cause infections, and are cost effective.

The present study explored the antimicrobial properties of the fruits of *Embelia ribes*,[7] the rhizome of *Nardostachys jatamansi,*[8] and the stolons of *Picrorhiza kurroa*.[9] All three are versatile medicinal plants with a rich history in traditional medicine, but supportive findings that underscore their therapeutic perspective are needed.

Embelia ribes, Vidanga or false black peppers, belong to the Myrsinaceae family.[10] In traditional practices, fruits of this herb are used as anthelmintics, carminatives, laxatives, and treatments for digestive disorders.[10] Recent studies have shown that this plant has antibacterial, anthelmintic, antidiabetic, anxiolytic[11] anti-inflammatory, and wound-healing properties.[12-15] The seed extract showed mild antibacterial activity against *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* but powerful antibacterial effects against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.[12] Studies have demonstrated that different extracts of *Embelia ribes* exhibit varying degrees of antibacterial activity against clinical isolates of multidrug-resistant bacteria.[15,16]

Nardostachys jatamansi is another medicinal plant commonly known as jatamansi.[8] Traditionally, rhizomes are used to treat neurological disorders, sleeplessness, anxiety disorders, and nootropes.[17] It is specifically useful in the treatment of hair loss and the enhancement of hair growth[18]; therefore, it is called bhoothajata (ghost hair) in Sanskrit, meaning that it promotes long hair growth. The rhizome of this plant has been studied for several pharmacological activities, such as hepatoprotective, cardioprotective, hypolipidemic,[19] and antifungal effects.[20] A study revealed that the hexane and n-butanol fractions of *Nardostachys jatamansi* exhibited a maximum zone of inhibition for *E. coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.[21]

Furthermore, this study explored the antimicrobial properties of another medicinal plant, *Picrorhiza kurroa,* or Katuki in Sanskrit. It is a hairy creeper commonly found in Himalayan regions.[9] The stolons of katuki plants are believed to have several medicinal uses, such as in the treatment of obesity, heart and liver disorders, hyperlipidemia, and diabetes,[22,23] Studies have shown that different extracts of Katuki have antibacterial and antifungal properties.[24,25]

Given the need for alternative therapeutic agents to treat multidrug-resistant bacterial infections, this research aimed to determine the antimicrobial potential of the above mentioned three medicinal herbs. These herbs can also be used as supplements to protect against infection, treat infection, or be taken along with standard antibiotics to potentiate their antibacterial effects. This study helps justify the use of these herbs in treating various chronic health conditions in traditional practices.

Aim and objectives

To study the antibacterial and antifungal potential of aqueous and ethanolic extracts

prepared from fruits of *Embelia ribes,* rhizomes of *Nardostachys jatamansi*, and stolons of *Picrorhiza kurroa.*

MATERIALS AND METHODS

Collection of fruits of *Embelia ribes*, rhizomes of *Nardostachys jatamansi* and stolons of *Picrorhiza kurroa*

Raw samples of herbs were collected from standard herbal drug suppliers in the Udupi district. Herbs were identified and confirmed by a botanist. The samples were cleaned well and allowed to dry in the dark for 15 days or until they became brittle. Then, all the dry samples were pounded separately into powders and preserved separately in airtight containers until they were used for extract preparation.[26]

Aqueous extract preparation

Dried and powdered samples prepared from fruits of *Embelia ribes,* rhizomes of *Nardostachys jatamansi* and stolons of *Picrorhiza kurroa* were used to prepare aqueous solutions with distilled water at a 1:10 ratio. The mixture was boiled in a low-speed flame until it was reduced to one-fourth. Once the mixture cooled to room temperature, the supernatant was separated and centrifuged at 3000 RPM for 5 minutes. The supernatant was separated and dried in a water bath. The dry extract obtained was stored in airtight containers.[26]

Ethanolic extract preparation

Ethanolic extracts were prepared via a Soxhlet apparatus. Dry powders prepared from fruits of *Embelia ribes,* rhizomes of *Nardostachys jatamansi*, and stolons of *Picrorhiza kurroa* were utilized for ethanolic extract preparation. The resulting solvent extract was stored in airtight containers at 4 °C.[26]

Antimicrobial activity

Standard antimicrobial strains obtained from the American Type Culture Collection (ATCC) were employed in this study included *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 90028). These strains were utilized to evaluate the antibacterial and antifungal activities of the aqueous and ethanolic extracts of the above selected herbs.[26]

Agar well diffusion technique

Bacterial and fungal cultures were initially revived by streaking onto nutrient agar and Sabouraud's dextrose agar (SDA), respectively. After incubation at 37 °C for 24 hours, distinct colonies were picked and inoculated into sterile Mueller–Hinton broth for bacterial strains, while *C. albicans* was subcultured in Sabouraud's dextrose broth. The inoculated broths were incubated overnight, and the microbial suspensions were standardized to approximately 1×10^5 CFU/ml in comparison with a 0.5 McFarland turbidity standard. Ampicillin (10 µg, micrograms) and amikacin (30 µg) served as the antibacterial reference drugs, whereas 10 µg of ketoconazole was used as the antifungal standard.[26]

To assess antibacterial activity

20 ml of Mueller-Hinton agar (MHA) was poured into each Petri dish. The bacterial culture was spread over the surface of the MHA plate. The agar was punched into 4 mm diameter wells, which were filled with 20 µl of solutions of the test compounds at various concentrations (100, 75, 50, 25, and 10 µg/ml). The inoculated plates were then incubated for 18 h at 37 °C. Tests were performed in triplicate, and the average of the three tests was considered for the study.[26]

Assessment of antifungal activity

Twenty millilitres of Sabouraud dextrose agar (SDA) were dispensed into sterile Petri dishes. A standardized culture of *C. albicans* was spread evenly across the agar surface. Wells 4 mm in diameter were made aseptically and filled with 20 µl of the test extracts at varying concentrations (100, 75, 50, 25, and 10 µg/ml). The inoculated plates were incubated at 37 °C for 18 hours. All experiments were carried out in triplicate, and the mean zone of inhibition values were recorded for evaluation.[26]

RESULTS

The antimicrobial efficacy of aqueous and ethanolic preparations of *Embelia ribes, Picrorhiza*

Table 1. Zone of inhibition of various bacterial and fungal strains by the aqueous extract of *Embelia ribes* (Vidanga)

Name of organism	Dosage of aqueous extract *Embelia ribes* (vidanga)(µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole*** 10 µg disc potency
Staphylococcus aureus (ATCC 25923)	25 mm	22 mm	17 mm	16 mm	6 mm	29 mm*
Enterococcus faecalis (ATCC 29212*)*	-	-	-	-	-	17 mm*
Escherichia coli (ATCC 25922)	-	-	-	-	-	20 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	19 mm**
Candida albicans (ATCC 90028)	7 mm	-	-	-	-	25 mm***

(-): Indicates no zone of inhibition. Standard drugs, *Ampicillin, ** amikacin, *** Ketoconazole.
µg - microgram, ml - millilitre, mm - millimeter

Table 2. Zone of inhibition of various bacterial and fungal strains by the ethanolic *Embelia ribes* (vidanga) extract

Name of organism	Dosage of ethanolic extract of *Embelia ribes* (vidanga)(µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole*** 10 µg disc potency
Staphylococcus aureus (ATCC 25923)	26 mm	24 mm	21 mm	8 mm	8 mm	29 mm*
Enterococcus faecalis (ATCC 29212)	15 mm	13 mm	7 mm	6 mm	6 mm	18 mm*
Escherichia coli (ATCC 25922)	-	-	-	-	-	22 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	20 mm**
Candida albicans (ATCC 90028)	-	-	-	-	-	28 mm***

(-): Indicates no zone of inhibition. Standard drugs, *Ampicillin, ** Amikacin, ***Ketoconazole.
µg - microgram, ml - millilitre. mm - millimeter

kurroa and *Nardostachys jatamansi* against various bacterial strains, such as *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and the fungal strain *Candida albicans,* was studied by measuring the zone of inhibition. The efficacies of these extracts were compared with those of the standard antibiotics ampicillin and amikacin and the antifungal agent ketoconazole.

The results revealed that the aqueous and ethanolic extracts of *Embelia ribes,* when used against *S. aureus*, presented a maximum zone of inhibition at different doses of extract. The results were comparable to those of the standard drug ampicillin (10 µg disc potency) when it was used against the same strain. The ethanolic extract of *Embelia ribes* also showed a maximum zone of inhibition against *Enterococcus faecalis* at dosages of 100 and 75 µg/ml. The effects were very similar to those of the standard antibiotic ampicillin (10 µg disc potency). This herb is not effective against *E. coli* or *Pseudomonas aeruginosa* strains and does not show antifungal activity when used against *Candida albicans* (Tables 1 and 2).

The aqueous and ethanolic extracts of *Picrorhiza kurroa,* when used against the *Staphylococcus aureus* strain, showed a maximum zone of inhibition at the 100, 75 and 50 µg/ml doses. The ethanolic extract also had a maximum zone of inhibition against *Enterococcus faecalis* at 100 and 75 µg/ml. The results were very similar to those of the standard antibiotic ampicillin when it was used against this strain. Both aqueous and ethanolic extracts of this herb did not affect *E. coli*

Table 3. Zone of inhibition of various bacterial and fungal strains by the aqueous extract of *Picrorhiza kurroa* (katuki)

Name of the organism	Dosage of aqueous extract *Picrorhiza kurroa* (katuki)(µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole***10 µg disc potency
Staphylococcus aureus (ATCC 25923)	16 mm	14 mm	13 mm	10 mm	9 mm	28 mm*
Enterococcus faecalis (ATCC 29212)						17 mm*
Escherichia coli (ATCC 25922)	-	-	-	-	-	20 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	19 mm **
Candida albicans (ATCC 90028)	-	-	-	-	-	24 mm***

(-): Indicates no zone of inhibition. Standard drugs, *Ampicillin, **Amikacin, ***Ketoconazole.
µg - microgram, ml - millilitre, mm - millimeter

Table 4. Zone of inhibition of various bacterial and fungal strains by the ethanolic extract of *Picrorhiza kurroa* (katuki)

Name of the organism	Dosage of ethanolic extract of *Picrorhiza kurroa* (katuki)(µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole***10 µg disc potency
Staphylococcus aureus (ATCC 25923)	18 mm	14 mm	14 mm	11 mm	10 mm	29 mm*
Enterococcus faecalis (ATCC 29212)	14 mm	12 mm	10 mm	9 mm	6 mm	20 mm*
Escherichia coli (ATCC 25922)	-	-	-	-	-	21 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	21 mm **
Candida albicans (ATCC 90028)	-	-	-	-	-	26 mm***

(-): Indicates no zone of inhibition. Standard drugs, * Ampicillin, **Amikacin, *** Ketoconazole.
µg - microgram, ml - millilitre, mm - millimeter

or Pseudomonas aeruginosa strains and did not have antifungal effects on *Candida albicans* strains (Tables 3 and 4).

Aqueous and ethanolic extracts of *Nardostachys jatamansi* rhizomes have been shown to have antimicrobial effects on *Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli* strains. The maximum zone of inhibition was observed at dosages of 100, 75 and 50 µg/ml, which is very similar to that of the standard antibiotic ampicillin when tested against the same organism. However, these compounds did not have any effect when tested against *Pseudomonas aeruginosa* and *Candida albicans* strains. These findings indicate that the extracts of *Nardostachys jatamansi* rhizome have antibacterial effects but no antifungal effects (Tables 5 and 6).

DISCUSSION

In this study, both aqueous and ethanolic extracts of *Embelia ribes* fruit, *Picrorhiza kurroa* stolon and *Nardostachys jatamansi* rhizomes were highly effective against *Staphylococcus aureus* at 100, 75 and 50 µg/ml. *Picrorhiza kurroa* stolon and *Nardostachys jatamansi* rhizome extracts have been shown to inhibit *Enterococcus faecalis* and *Escherichia coli* strains. *Nardostachys jatamansi* rhizome extracts were effective against all three abovementioned bacterial strains. *Staphylococcus*

Table 5. Zone of inhibition of various bacterial and fungal strains by the aqueous extract of *Nardostachys jatamansi* (jatamansi)

Name of the organism	Dosage of aqueous extract *Nardostachys jatamansi* (µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole*** 10 µg disc potency
Staphylococcus aureus (ATCC 25923)	28 mm	26 mm	22 mm	20 mm	17 mm	28 mm*
Enterococcus faecalis (ATCC 29212)	21 mm	18 mm	16 mm	11 mm	7 mm	18 mm*
Escherichia coli (ATCC 25922)	16 mm	15 mm	14 mm	8 mm	6 mm	20 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	19 mm **
Candida albicans (ATCC 90028)	-	-	-	-	-	24 mm***

(-): Indicates no zone of inhibition. Standard drugs, *Ampicillin, **Amikacin, *** Ketoconazole.
µg - microgram, ml - millilitre, mm - millimeter

Table 6. Zone of inhibition of various bacterial and fungal strains by the ethanolic extract of *Nardostachys jatamansi* (jatamansi)

Name of the organism	Dosage of ethanolic extract *Nardostachys jatamansi* (µg/ml)					Standard antibiotics & antifungal drugs
	100	75	50	25	10	Ampicillin*10 µg Amikacin** 30 µg Ketoconazole*** 10 µg disc potency
Staphylococcus aureus (ATCC 25923)	29 mm	27 mm	25 mm	22 mm	19 mm	29 mm*
Enterococcus faecalis (ATCC 29212)	22 mm	20 mm	19 mm	14 mm	13 mm	20 mm*
Escherichia coli (ATCC 25922)	20 mm	18 mm	16 mm	10 mm	8 mm	21 mm*
Pseudomonas aeruginosa (ATCC 27853)	-	-	-	-	-	21 mm **
Candida albicans (ATCC 90028)	-	-	-	-	-	26 mm***

(-): Indicates no zone of inhibition. Standard drugs * Ampicillin, **Amikacin, ***Ketoconazole.
µg - microgram, ml - millilitre, mm - millimeter

aureus bacteria can cause a wide range of infections in humans.

Although these extracts alone are not effective at inhibiting fungi, they are the ingredients in the formulations that are used to treat fungal infections. The Ayurvedic literature indicates that herbal preparations produce greater therapeutic effects when used in combination rather than individually. Ayurvedic medicines are usually made by mixing several herbs, where one main herb takes the lead. Together, these herbs work in synergy to make the treatment more effective. The medicinal properties of herbs are due to the presence of active components,[27] their antioxidant capacity,[28] and their immune-modulating effects.

Embelia ribes are a key ingredient in Vidangarista, a fermented Ayurveda preparation used against intestinal parasites.[29] Similarly, *Nardostachys jatamansi* essential oil or Jatamansi oil is used by traditional practitioners for the treatment of skin diseases and dandruff.[30] Dashangalepa is one of the ingredients in Ayurvedic preparations and is used to treat skin infections. It has various important phytochemicals[31] that are responsible for medicinal and antioxidant properties.[32] The study revealed that the n-Butanol fraction present in this herb is a powerful

inhibitor of all pathogens, especially *Escherichia coli.*[21] Furthermore, *Picrorhiza kurroa* powder is commonly advised for the treatment of respiratory infections. It contains seven iridoid glycosides[33]; many other active constituents have also been identified, and some still unidentified substances have been identified.[34] These findings suggest that the herbs selected in this study have the potential to act against various infections, particularly when they are incorporated into herbal formulations, where their efficacy is enhanced through synergistic interactions with other constituents.

CONCLUSION

The results of the present study show that all three herbs have antibacterial effects, among which *Nardostachys jatamansi* has more effective antimicrobial effects than the other two herbs do. The results of this study suggest a path for the development of new antimicrobial therapies, particularly in the context of increasing resistance to antibiotics. The diverse mechanisms of action and traditional use of these herbs in medicine highlight their potential as valuable resources in modern pharmacotherapy. Advanced studies are essential for isolating phytocompounds that are reliable for antimicrobial activity. Continued research into its antimicrobial properties and mechanisms will be essential for fully realizing its therapeutic potential.

ACKNOWLEDGMENTS

The authors are grateful to Manipal Academy of Higher Education for supporting the research work and providing the facility to conduct this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PMDA designed the study, collected the herbs and prepared the herbal powders. KSRP prepared the aqueous and ethanolic extracts of all the three herbs. KLS performed the experiment. PMDA and KLS collected data and performed data analysis. PMDA wrote the manuscript. KLS and KSRP critically reviewed the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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