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ANTIBACTERIAL POTENTIAL OF DIFFERENT EXTRACTS OF VARIOUS PARTS OF *JUSTICIA ADHATODA* L.

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In Ayurvedic and Unani medicine, *Justicia adhatoda* is a well-known medicinal herb. It is commonly known that higher plants contain antibacterial agents. The antimicrobial potential of *Justicia adhatoda*, alone makes a major contribution. An indicator of an antimicrobial agent's relative potential is the formation of a growth inhibition zone, which is a clear area surrounding the original agent and indicates the efficacy of the agent. The present study aimed to evaluate antimicrobial activity of at different concentrations (25, 50, 75 & 100 µg/ml) of methanol, pet ether and water extract of leaf, stem, root and flower part of *J. adhatoda* against Gram-negative (*E. coli* and *P. syringe*) and gram-positive (*B. cereus* and *S. aureus*) bacterial strains. Antibiotic was used as standard. Based on the data presented here in; for root, the largest zone of inhibition was found to be against *B. cereus* (13mm) in water extracts at 100 µg/ml concentration followed by against all other three bacteria with 12mm inhibition zone in methanol extract. For stem, the largest zone of inhibition was found to be against *E. coli* (17mm) in methanol extracts followed by against *S. aureus* with 16mm inhibition zone in methanol and water extracts (100 µg/ml concentration). In case of flower, the maximum inhibition Zone (IZ) was 17 mm against *E. coli* (in water extract) and *S. aureus* (in methanol extract) followed by *P. syringe* (methanol extract) with 15mm inhibition zone. For leaves, highest IZ was 17 mm against *E. coli* (in water extract) followed by *S. aureus* (in methanol and pet ether extract) with IZ of 15mm. So, this study provided referential information about the antimicrobial activity of different extracts of different parts of *Justicia adhatoda* L. It may be effective in identifying a new bioactive compound for the development of novel medications.

Keywords: Antimicrobial activity, *B. cereus, E. coli, Justicia adhatoda, P. syringe* and *S. aureus*.

Introduction

Traditional healthcare is based on plants with medicinal properties, which have been the focus of much pharmacological study in the past few years. Because they are a source of healing compounds, higher plants have controlled human health since the dawn of time. Natural goods are the source of more than half of all contemporary clinical medications, and they are crucial to pharmaceutical companies' drug development initiatives[1]. Medicinal plants are thought to be a significant source of novel compounds for drug development as well as a possible source of novel compounds with therapeutic applications[2]. In order to defend themselves against bacteria and fungi, these plants produce a variety of chemical substances that function on the human body similarly to allopathic

medications[3,4,5]. A significant public health issue is the worldwide incidence of bacterially-caused infectious illnesses[6]. Due to their similar safety and efficacy, plants are being studied for their potential as antimicrobial substances against resistant strains as a result of the recent growth of antibiotic resistance and associated toxicity challenges, which constrain the effective application of antimicrobial compounds[7,8]. In nations with limited resources, between 60 and 90 percent of the populace takes medicine obtained from plants. Crude plant extracts have historically been utilized as herbal medicine to treat viral disorders in humans[9]. Numerous phytochemicals isolated from plants, such as flavonoids, terpenoids, alkaloids, and tannins, have been shown to have antibacterial qualities. These formulations mediate significant host responses, even though the mechanism of action and efficacy of these herbal extracts in most situations remain required to be verified scientifically[10,11].

Justicia adhatoda (malabar nuts), belonging to family Acanthaceae, is well-known in India as a medicinal herb that is used extensively in homoeopathy, Siddha, Ayurveda, Unani, and other traditional medical practices[12]. *J. adhatoda* has been used so frequently that it has been included in the WHO handbook "The Use of Traditional Medicine in Primary Health Care," which is meant to enlighten healthcare professionals on the healing potential of the plants in their immediate environment[13]. The primary compounds that make up *Justicia adhatoda* include vasicine, quinazoline alkaloids, vasicinone, and vasicine. Phytosterols, glycosides, and polyphenols are also prevalent. The essential oil obtained from the leaves of *Justicia adhatoda* contained a long list of chemical substances, which includes phytosterols, anthraquinones, alkaloids, polyphenols, flavonoids, saponins, and triterpenoids comprising N-oxides of vasicine, vasicine, maiontone, and deoxyvasicine[14]. Leaf, root, stem, and flower parts of Adhatoda are used in a variety of pharmaceutical formulations. Their extracts are employed as sedative expectorants in herbal treatments for a variety of ailments, including diarrhoea, dysentery, painful rheumatic inflammatory swellings, whooping cough, fever, cold, cough, jaundice, and asthma[11]. Since it contains these chemical substances, this plant demonstrates a wide range of biological attributes, such as anti-diabetic, antibacterial, anti-inflammatory, anti-malarial, anti-oxidant, anti-mutagenic, respiratory stimulant, and bronchodilatory activities; additionally, it demonstrates potentials for cardio-protection, anti-ulcer, insecticidal, hepato-protection, and anti-cholinesterase actions[14,15]. Therefore, the present study investigated the antimicrobial activity of different extracts of different parts of *Justicia adhatoda* against bacteria such as *E. coli, P. syringe*, *B. cereus* and *S. aureus*.

Material and Methods

Collection and extraction of plant materials: Root, stem, leaves and flowers of *Justicia adhatoda* collected from Jaipur, Rajasthan. Those were washed with distilled water and then air dried at room temperature. After complete drying plant materials were grinded to make fine powder and stored for further use.

1 gm of the grinded plant material was dissolved in 10 ml of water (polar), methanol (mid-polar) and pet ether (non-polar) solvents and kept at shaker for 48 hours at room temperature. After that, all were filtered, and solvent was evaporated to obtain dry extract. Extractive values were calculated as mg/g.dw for each.

Evaluation of antibacterial activity: Standard microbial technique - the Agar Well Diffusion method[16]was used for *in-vitro* antimicrobial assay. For antibacterial

activity, Nutrient agar was used. The different samples were diluted by using dimethyl sulphoxide (DMSO) and 4 different concentrations (25 mg/L, 50 mg/L, and 75 mg/mL and 100 mg/mL) of all compounds were prepared. Disinfected Petri-dishes holding the nutrient agar (NA) medium were used for the inoculation of test microorganisms, this inoculums spread all over the dish using spreader and kept standing for 30 min. Wells of 6 mm diameter were prepared in the seeded agar plates. In a different Petri-plate, standard drug was also loaded at similar concentrations. All different concentrations of all the samples and standard drug (30 µl in each well) poured into the preorganized wells of seeded plates. The plates were kept for incubation at 37ºC for 24 hrs. The antibacterial spectrum of the test sample was determined via inhibition zone (IZ) around each prepared well. The comparison of diameters of inhibition zone developed by the test sample and by the commercial control antibiotic (streptomycin) was done.

Activity Index was calculated from comparison of activity of samples with the standard antibiotic drug. The activity index was calculated by the following formula-

Activity Index (AI) = Inhibition zone of sample/Inhibition zone of standard

Result and Discussion

Antibacterial activity of different parts of Justicia adhatoda L. against E. coli

The results in Table 1 depict antibacterial activity of different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of root, stem, leaf and flower extract of *Justicia adhatoda* in different solvents (water, methanol and Pet. Ether) against *E. coli*. Results indicate potent antibacterial efficacy of all tested extracts against *E. coli* in a dose dependent manner, with maximum antibacterial efficacy in case of flower extract followed by leaf, root and stem extract. Maximum antibacterial efficacy in root extract was obtained in case of methanol followed by almost equal antibacterial activity in water and Pet Ether. Maximum antibacterial efficacy in stem extract was obtained in case of methanol followed by Pet Ether and water. In case of leaves, maximum antibacterial activity was observed in case of water followed by methanol and Pet Ether. Similar results were obtained in case of flower extract, where too, maximum antibacterial activity was obtained in case of water followed by Pet ether and methanol. No antibacterial activity was displayed by lowest dose of methanolic flower extract.

A recent study revealed phytochemical profile of different plant parts of *Justicia adhatoda* L. (leaves, flowers, stem and roots) and showed presence of maximum phenolic and flavonoids content in flowers of the plant in comparison to other plant parts. Therefore, the highest antibacterial efficacy of flower extract in the current study against the test pathogen *E. coli* maybe attributed to comparatively higher phenolic and flavonoid content[17]. Differential antibacterial efficacy of extracts in different solvents maybe due to differential rate of extraction of the phytochemicals due to different solvent polarities. Similar results have been shown in another recent study**,** wherein differential antibacterial activity of *Justicia adhatoda* leaf extract in different solvents (n-hexane, water, methanol and ethanol) against four bacterial species (*E. coli, S. aureus, Pseudomonas fluorescens* and *Bacillus subtilis)* was attributed to differential extraction yield[18]. The antibacterial efficacy of Methanolic, ethanolic, and ethyl acetate crude extracts of *Justicia adhatod* have also been reported against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* and attributed to presence of bioactive secondary

metabolites namely, saponins, tannins, alkaloids, steroids, phenols, and flavonoids[19]. Similar results have been reported in another recent study demonstrated antibacterial, antifungal, anti-inflammatory, and antioxidant activities displayed by ethanolic leaf extracts of *Justicia adhatoda* L against *Escherichia coli* (MTCC 82), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453), and *Klebsiella pneumonia* (MTCC 39). The antibacterial activity of the tested plant species was attributed to presence of pharmacologically important compounds, viz. 1-hexyl-2-nitrocyclohexane, 2-naphthalenamine, 1-butanol, 3-methyl-, acetate, and 1-docosene[20].

Table 1: Antibacterial activity of different extracts of different parts at various concentrations of *Justicia adhatoda* against *E. coli.*

		E.coli							
Concentrations (µg/ml)		**25 mg/L**		**50 mg/L**		**75 mg/L**		**100 mg/L**	
Plant Parts	**Extracts**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**
Root	Water	7	0.291667	8	0.266667	9	0.290323	10	0.3125
	Methanol	8	0.333333	10	0.333333	11	0.354839	12	0.375
	Pet. Ether	7	0.291667	8	0.266667	10	0.322581	11	0.34375
Stem	Water	8	0.333333	11	0.366667	13	0.419355	16	0.5
	Methanol	8	0.333333	10	0.333333	14	0.451613	17	0.53125
	Pet. Ether	7	0.291667	10	0.333333	12	0.387097	15	0.46875
Leaves	Water	8	0.333333	10	0.333333	15	0.483871	17	0.53125
	Methanol	7	0.291667	8	0.266667	10	0.322581	11	0.34375
	Pet. Ether	7	0.291667	8	0.266667	9	0.290323	12	0.375
Flower	Water	11	0.458333	13	0.433333	16	0.516129	17	0.53125
	Methanol	NA	NA	7	0.233333	9	0.290323	10	0.3125
	Pet. Ether	8	0.333333	9	0.3	11	0.354839	13	0.40625
Standard		24		30		31		32	

Antibacterial activity of different parts of Justicia adhatoda L. *against S. aureus:* The results in Table 2 depict antibacterial activity of different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of root, stem, leaf and flower extract of *Justicia adhatoda* in different solvents (water, methanol and Pet. Ether) against *S. aureus.* Results indicate potent antibacterial efficacy of all tested extracts against *S. aureus* in a dose dependent manner, with maximum antibacterial efficacy in case of flower extract followed by leaf, stem and root extract. Maximum antibacterial efficacy in root extract was obtained in case of methanol followed by almost equal antibacterial activity in water and Pet Ether. Maximum antibacterial efficacy in stem extract was obtained in case of methanol followed by Pet Ether and water. In case of leaves, maximum antibacterial activity was observed in case of water followed by methanol and Pet Ether. No antibacterial activity was displayed by low doses of water flower extract. Similar results were obtained in case of flower extract, where too, maximum antibacterial activity was obtained in case of methanol followed by Pet ether and water.

Antibacterial efficacy of different extracts of *Justicia adhatoda* L. against *S. aureus* is attributed to presence of a number of bioactive secondary metabolites, namely flavonoids, phenolics, saponins, tannins and others, all of which have been reported to act as anti-inflammatory, antioxidant, anti-

microbial and antifungal in nature. Similar results, showing antibacterial efficacy of *Justicia adhatoda* L. against *S. aureus* has been reported in a number of recent studies[21-23]

Antibacterial activity of different parts of Justicia adhatoda L. against Pseudomonas syringae

The results in Table 3 depict antibacterial activity of different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of root, stem, leaf and flower extract of *Justicia adhatoda* in different solvents (water, methanol and Pet. Ether) against *Pseudomonas syringae.* Results indicate potent antibacterial efficacy of all tested extracts against *Pseudomonas syringae* in a dose dependent manner, with maximum antibacterial efficacy in case of flower extract followed by leaf, root and stem extract. Maximum antibacterial efficacy in root extract was obtained in case of methanol followed by almost equal antibacterial activity in water and Pet Ether.

Maximum antibacterial efficacy in stem extract was obtained in case of Pet Ether followed by water and methanol. In case of leaves, maximum antibacterial activity was observed in case of Pet Ether followed by water and methanol. Similar results were obtained in case of flower extract, where too, maximum antibacterial activity was obtained in case of methanol followed by water. No antibacterial activity was displayed by lowest dose of Pet ether flower extract. The antibacterial activity of *Justicia adhatoda* L. against *Pseudomonas syringae* maybe attributed to presence of bioactive compounds, namely, flavonoids, phenolics, saponins and tannins. However, till date, only a few studies have reported the antibacterial activity of *Justicia adhatoda* L. against *Pseudomonas syringae,* wherein, the researchers isolated a soil bacterium and identified it to be *Pseudomonas syringae*. Thereafter, antibacterial efficacy of different plant extracts including *Justicia adhatoda* was reported against *Pseudomonas syringae*[24]. However, a number of studies have reported antibacterial efficacy of *Justicia* against another species of *Pseudomonas*, namely *Pseudomonas syringae*[25-27].

Table 2: Antibacterial activity of different extracts of different parts at various concentrations of *Justicia adhatoda* L. against *S. aureus.*

		Staphylococcus aureus							
Concentrations (µg/ml)→		**25 mg/L**		**50 mg/L**		**75 mg/L**		**100 mg/L**	
Plant Parts	**Extracts**	**IZ (mm**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**
Root	Water	7	0.225806	8	0.228571	10	0.277778	11	0.289474
	Methanol	8	0.258065	9	0.257143	10	0.277778	12	0.315789
	Pet. Ether	7	0.225806	8	0.228571	9	0.25	10	0.263158
Stem	Water	9	0.290323	10	0.285714	11	0.305556	16	0.421053
	Methanol	7	0.225806	13	0.371429	14	0.388889	16	0.421053
	Pet. Ether	8	0.258065	9	0.257143	11	0.305556	14	0.368421
Leaves	Water	NA	NA	NA	NA	8	0.222222	10	0.263158
	Methanol	7	0.225806	10	0.285714	13	0.361111	15	0.394737
	Pet. Ether	8	0.258065	12	0.342857	14	0.388889	15	0.394737
Flower	Water	7	0.225806	8	0.228571	15	0.416667	16	0.421053
	Methanol	11	0.354839	13	0.371429	15	0.416667	17	0.447368
	Pet. Ether	7	0.225806	8	0.228571	13	0.361111	15	0.394737
Standard		31		35		36		38	

Table 3: Antibacterial activity of different extracts of different parts at various concentrations of *Justicia adhatoda* L. against *P. syringe.*

Pseudomonas syringe

Concentrations (µg/ml) →		25		50		75		100	
Plant Parts	**Extracts**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**
Root	Water	6	0.272727	7	0.30434783	8	0.296296	9	0.333333
	Methanol	8	0.363636	9	0.39130435	11	0.407407	12	0.444444
	Pet. Ether	8	0.363636	9	0.39130435	10	0.37037	12	0.444444
Stem	Water	7	0.318182	8	0.34782609	10	0.37037	11	0.407407
	Methanol	7	0.318182	8	0.34782609	9	0.333333	11	0.407407
	Pet. Ether	7	0.318182	9	0.39130435	11	0.407407	14	0.518519
Leaves	Water	7	0.318182	8	0.34782609	9	0.333333	10	0.37037
	Methanol	7	0.318182	8	0.34782609	11	0.407407	13	0.481481
	Pet. Ether	7	0.318182	8	0.34782609	11	0.407407	14	0.518519
Flower	Water	7	0.318182	8	0.34782609	9	0.333333	11	0.407407
	Methanol	9	0.409091	10	0.43478261	14	0.518519	15	0.555556
	Pet. Ether	NA	NA	NA	NA	7	0.259259	8	0.296296
Standard		22		23		27		27	

Antibacterial activity of different parts of Justicia adhatoda L. against B. cereus

The results in Table 4 depict antibacterial activity of different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of root, stem, leaf and flower extract of *Justicia adhatoda* in different solvents (water, methanol and Pet. Ether) against *B. cereus*. Results indicate potent antibacterial efficacy of all tested extracts against *B. cereus* in a dose dependent manner, with nearly same antibacterial efficacy in case of all the tested extracts. Maximum antibacterial efficacy in root extract was obtained in case of water followed by almost equal antibacterial activity in methanol and Pet Ether. Maximum antibacterial efficacy in stem extract was obtained in case of methanol followed by Pet Ether and water. In case of leaves, maximum antibacterial activity was observed in case of methanol followed by water and Pet Ether. Similar results were obtained in case of flower extract, where too, maximum antibacterial activity was obtained in case of water followed by Pet ether and methanol. Similar results have been reported in a recent study, where too, the researchers reported antibacterial efficacy of leaf extract of *Justicia adhatoda* as well as green silver nano-particles synthesized from *Justicia adhatoda* against *Escherichia coli, Klebsiella aerogens, Staphylococcus aureus, Salmonella typhi and Bacillus cereus*[28]. In another study, researchers attempted to synthesize an antioxidant and antibacterial liquid soap from methanolic extract of *Justicia adhatoda*[29]. The antibacterial efficacy of this liquid soap extract was tested against *B. cereus, S. typhimurium, S. aureus* and *E. coli* and its antibacterial activity was attributed to the presence of bioactive secondary metabolites. The studies showing antibacterial efficacy of *Justicia adhatoda* against *Bacillus cereus* are very limited. However, a number of studies have

reported antibacterial efficacy of *Justicia adhatoda* against another *Bacillus* species, namely, *Bacillus subtilis*[19, 23].

Conclusion

The antimicrobial activities may be due to the strong occurrence of active compounds, i.e., saponins, tannins, alkaloids, steroids, phenols, and flavonoids. It has been concluded that *Justicia adhatoda* extracts are suitable candidates for the development of novel antibacterial compounds. The current study was aimed at the evaluation of *Justicia adhatoda* L. extracts for their antibacterial potential. The results showed that different parts of *Justicia adhatoda* L. have considerable antibacterial action against both the selected gram-negative (*E. coli* and *P. syringe*) and gram-positive (*B. cereus* and *S. aureus*) microorganisms. Extracts of Stem was found to have more potential followed by extracts of flowers, leaves and roots. All the extracts were great in their antibacterial activity. Further phytochemical analysis of these plant parts will be helpful for the elucidation of lead molecules an it may be employed as an eco-friendly, biodegradable alternative to prevent and treat bacterial infections.

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