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Antibacterial Activity of *Justicia betonica* Linn.

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Abstract

Justicia betonica L., (Acanthaceae) is widely used as a traditional folk medicinal herb. Conventionally, the plant is used to cure constipation, diarrhea, malaria, pain, stomach ache, vomiting, etc. The plant possesses many therapeutic uses such as analgesic, anti-inflammatory, antimalarial, and antimicrobial properties. The present work has been designed to document the ethnomedicinal properties, phytochemistry, and antibacterial activity of *J. betonica*. Phytochemical results showed that the presence of wide variety of biologically active compounds such as flavonoids, alkaloids, tannins, and phenolic compounds. Antibacterial activity showed that the inhibition was observed with the individual extracts and was effective against all bacterial strains tested. The present study highlights the importance of *J. betonica*.

Keywords: Acanthaceae, Antibacterial activity, Ethnomedicinal, Phytochemistry

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Introduction

Acanthaceae consists of about 4,300 species belonging to 346 genera and top 12 most diverse families.[1] These plants are horticulturally important and cultivated as ornamental plant.[2,3] Characteristic features of Acanthaceae are opposite phyllotaxy of leaves and usually leaves with entire margin, stems are round to quadrangular with solitary or racemose inflorescence, bisexual flowers, large and petaloid bracts, 4–5 petals and sepals, 2–4 stamens, and superior ovary with two fused ovules. The fruit is often explosively dehiscent present inside loculicidal capsule. Seeds are usually borne on hook such as retinacula, or retinacula lacking, surface smooth or roughened lacking trichomes or pubescent, and sometimes with hygroscopic trichomes that expand when moistened.[4,5] *Justicia* is an important genus of Acanthaceae, with approximately 700 species, with many unresolved species which are found in pantropical and tropical regions.[6] *Justicia* species are reported to occur in tropical to warm temperate regions of America, India, Indonesia, Southeast Asia, Malaysia, Pakistan, and Africa.[7] The genus *Justicia* is characterized by plants usually herbs or under shrubs with simple lanceolate leaves and sessile or sub sessile flowers in spikes or panicles.[8] *Justicia betonica* Linn. belonging to this genus, is a diffusely branched under shrub or an erect shrub. This plant is native to tropical Asia and tropical Africa and is distributed throughout India and Sri Lanka. In Kerala, the species is common and widely distributed in Western Ghats usually found along forest margins.[9,10]

Various parts of this plant have been used as traditional Ayurvedic medicine in India as well as in other countries. The aerial part of this plant is used in diarrhea, inflammation, and swelling.[11] In India, the inflorescence extract of this plant is given orally to treat vomiting and constipation and used externally to wash hairs.[12] Leaves are crushed and applied to relieve pain and swelling.[13] Leaf and flower ash internally used for the treatment of cough, diarrhea etc.[14] Leaf decoction is used to cure vomiting and headache. In Uganda, the leaves of *J. betonica* are used against HIV/AIDS. *J. betonica* is administered to lower cholesterol and is used to treat paralysis, ear aches, headaches, bruises, diarrhea, vomiting, constipation, pain and inflammation, and malaria in India.[15] The plant possesses analgesic, antimalarial, antimicrobial, antioxidant, and anti-inflammatory properties.[9,10]

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Keeping the importance of *Justicia* species, the present study was designed. It may support in standardization of plant material which could give a hand in ascertaining identity and purity of crude drugs from the present study.

Methodology

Collection of Plant for Experimental Works

The sample was collected and kept in poly bags tagged with the botanical name and sorted out as per standard sampling procedure and passport description.[16]

Preparation of Extracts

Soxhlet method and percolation were adopted to obtain different extracts.[17,18] The collected experimental plant materials were dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residue was collected and left for air drying and dried crude extracts were stored in refrigerator for further phytochemical analysis and antibacterial activities.

Phytochemical Assays

Phytochemical analysis was carried out on different extracts of the whole plant of *J. betonica* using standard procedure to identify the bioactive compounds.[17,19,20]

Test for Tannins

To 5 ml of plant extract 5 drops of 10% lead acetate was added. Formation of a light yellow precipitate indicated the presence of tannins.

Test for Saponins

1 ml of the extract was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. Occurrence of stable persistent froth indicated the presence of saponins.

Test for Flavonoids

To 1 ml of the extract, few drops of dilute sodium hydroxide were added. The presence of flavonoids is indicated on production of an intense yellow color in the plant extract which became colorless on addition of 2–3 drops of 50 % HCL.

Test for Terpenoids

0.5 g of plant extract was mixed with 2 ml of chloroform and equal volume of concentrated sulfuric acid was added. Terpenoid presence is confirmed by a reddish brown coloration of interface.

Test for Phenolic Compounds

5 drops of 1% ferric chloride and 1 ml of potassium ferro cyanide were added to 2 ml of plant extract. A bluish green solution showed the presence of phenolic compounds.

Test for Reducing Sugar

0.5 g of plant extract was dissolved with distilled water and filtered. The filtrate was boiled with 2 drops of Fehling's solution A and B for 5 min. An orange red precipitate obtained indicated the presence of reducing sugar.

Test for Steroids

2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulfuric acid was added. Formation of two phases (upper red and lower yellow with green fluorescence) indicated the presence of steroid.

Test for Alkaloids

5 ml of plant extract was mixed with 3 ml of aqueous HCL on water bath and then filtered. 1 ml of Dragendorff's was added to the filtrate. The occurrence of orange red precipitate indicated the presence of alkaloids in the sample extract.

Test for Carbonyl

2 ml of plant extract was added with two drops of 2, 4-dinitrophenyl hydrazine solution and thoroughly shaken. Yellow crystal formation indicated the presence of carbonyl.

Antibacterial Activity

The extracts of experimental plant were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* Microbial Type Culture Collection (MTCC, 497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), and *Shigella flexneri* (MTCC 1457). All used MTCC bacterial strains were collected from Institute of Microbial Technology, Chandigarh. Antibacterial activity was done using slight modification of standard methods of agar well diffusion assay;[21] disk diffusion method;[22] and broth dilution assay.[23]

Agar Well Diffusion Assay

Agar well diffusion method[21] was followed to test the antibacterial activity of extracts of experimental plant parts against five bacterial strains. Nutrient agar plates were prepared as per instructions of manufacturer. 100 µl of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (Sigma) and two-fold serial dilutions were made in amount of 100 µl per well at concentration of 0.25 and 0.5 mg/ml. 100 µl of samples were added by sterile syringes into the wells in three above-mentioned concentrations and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18–24 h. Kanamycin served as standard antibiotic control. Triplicates were maintained and the experiment was repeated thrice. For each replicate, the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Disk Diffusion Assay

Antibacterial activity using disk diffusion assay was done using the 6 mm of disk prepared from Whatman filter paper.[22] Each extract was dissolved in dimethyl sulfoxide. The sets of dilutions (10 µg/disc and 50 µg/disc) of crude extracts and standard drugs were prepared. 6 mm disks were kept in the drugs for 12 h before placing onto the agar plates. The zones of growth inhibition around the disks were measured after 18–24 h of incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

Minimum Inhibitory Concentration (MIC) Using Broth Dilution Assay

All the extracts of experimental plant were screened for their antibacterial activity (modified method).[23] Antibacterial activity was assessed by MIC by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8 ± 1 h at 35 ± 2°C until there was a visible growth. Mc Farland No.5 standard and PBS (Phosphate Buffer Saline) were used to adjust the turbidity to get 105 cfu/mL.

Table 1: Phytochemical test of selected experimental plants (*Justicia betonica*)

Plant Name	*Bioactive Compounds*	*Solvents*					
		n-hexane	*Petroleum ether*	*Methanol*	*Aqueous*	*Acetone*	*Ethanol*
Justicia betonica (Whole plant)	Tannin	-	-	+++	-	+++	+++
	Saponin	-	-	-	-	-	-
	Flavonoids	-	-	+++	++	-	-
	Terpenoids	-	-	-	-	-	-
	Phenolic compounds	-	-	+++	+++	+++	+++
	Reducing sugar	+++	-	+++	-	+++	-
	Steroid	-	-	-	-	-	-
	Alkaloids	-	-	+++	+++	+++	-
	Carbonyl	-	-	-	-	-	-

+++: High concentration, ++: Mild concentration, +: Low concentration

Data Interpretation

After the incubation, the tubes showing no visible growth after 8 h till 12 h were considered to be inhibition of bacteria which represent MIC (minimum inhibitory concentration) values of a respective concentration. Inoculum control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to absence of bacteria. Triplicates were maintained and the experiments were repeated thrice for each replicate. The readings were taken as foresaid.

Results

The present study is based on both field and laboratory works. The crude powder of the collected experimental plant (*J. betonica*) was extracted using Soxhlet method. The extracts were used in the qualitative analysis of secondary metabolites, antimicrobial activity using agar well diffusion, disk diffusion, and MIC assay.

Phytochemical Screening

An attempt has been made to evaluate the presence of bioactive compounds in the experimental plant through qualitative tests. The different extracts of *J. betonica* were analyzed to study the presence of secondary metabolites. The phytochemical screening revealed the presence of diverse secondary metabolites which are of highly pharmacological significance. The common secondary metabolites are present in the extracts are tannins, alkaloids, flavonoids, and phenolic compounds [Table 1].

Evaluation of Antimicrobial Activity

Agar well diffusion

Antimicrobial activity of different extracts of the experimental plant (*J. betonica*) was determined against two Gram-positive bacteria *S. mutans* (MTCC 497) and *S. pyogenes* (MTCC 1926); three Gram-negative bacteria *V. cholerae* (MTCC 3906), and *S. flexneri* (MTCC 1457). The results revealed that the methanolic extract showed the highest zone of inhibition against all the pathogens [Table 2].

Disk diffusion assay

Disk diffusion assay was carried out and it was examined that the zone of inhibition was aqueous, methanol, and acetone extract for all the pathogens. It was observed that the highest zone of inhibition in aqueous extract was 11.5 mm against *S. flexneri*. Methanol, acetone, ethanol, and n-hexane extracts showed zone of inhibition of 11.0 mm, 9.2 mm, 8.1 mm, and 8.5 mm, respectively, against *V. cholerae* [Table 3].

Table 2: Antibacterial activity of *Justicia betonica* using Agar Well Diffusion method

Extracts	*Zone of Inhibition (mm)*			
	Streptococcus mutans	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous	8.5	8.0	10.6	10.5
Methanol	10.8	10.3	12.1	11.5
Acetone	9.5	9.2	10.5	10.8
Ethanol	8.2	8.1	9.5	9.3
n-hexane	8.0	8.0	9.2	9.4

Table 3: Antibacterial activity of *Justicia betonica* using disk diffusion method

Extracts	*Zone of Inhibition (mm)*			
	Streptococcus mutans	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous	8.5	10.5	9.5	11.5
Methanol	9.3	10.2	11.0	9.5
Acetone	8.2	8.3	9.2	9.0
Ethanol	7.2	7.5	8.1	8.0
n-hexane	7.0	7.2	8.5	8.0

Table 4: Broth dilution assay of *Justicia betonica*

Extracts	*Minimum Inhibitory Concentration (mg/ml)*			
	Streptococcus mutans	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous	500	500	400	400
Methanol	400	400	350	350
Acetone	400	400	350	350
Ethanol	450	450	400	400
n-hexane	Growth	Growth	Growth	Growth
Inoculum	Growth	Growth	Growth	Growth
Broth	No growth	No growth	No growth	No growth

Broth dilution assay

Broth dilution was carried out using different extracts to determine the MIC values of all the extracts against *S. mutans*, *S. pyogenes*, *V. cholerae*, and *S. flexneri*. The result of minimum inhibitory concentration in aqueous, methanol, acetone, and ethanol extract was 400 mg/mm and 350 mg/mm against *V. cholerae* and *S. flexneri* [Table 4].

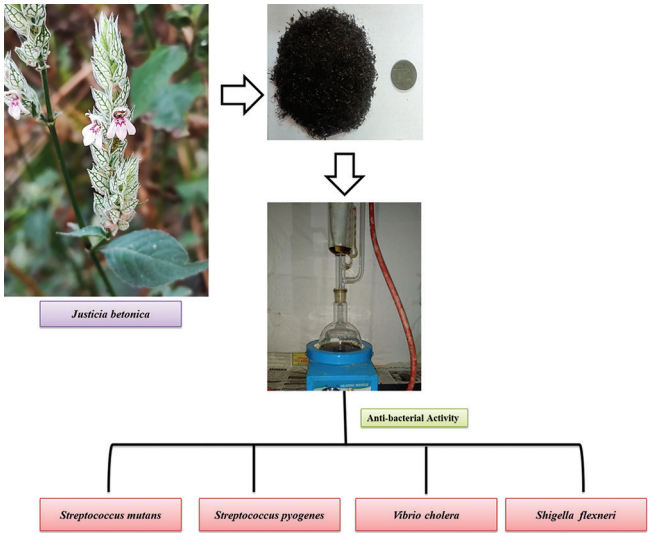

Figure 1: Plant extracts have great potential as antimicrobial compounds against microorganisms

Discussion

J. betonica plays a significant role in folk as well as in veterinary medicines but, it is a less explored plant species as compared to its traditional usefulness as well as its antibacterial activity. Sasikumar *et al.* in 2007 reported that the extract of *J. betonica* failed to inhibit the growth of *Klebsiella pneumonia*, *Salmonella* Typhi, and *V. cholerae*. The leaf extract of this plant is reported to be active against rice moth, Coraryra.[24] Ssenyondo *et al.* in 2020 reported that the leaf extract of *Justicia betonia* shows antimicrobial activity against *S. aureus*.[25] According to Correa and Alcantara 2012, lignans are the major components of the active extracts of the species of Justicia, exhibiting important pharmacological properties, such as antiviral, antitumoral, anti-inflammatory, and antiplatelet aggregation activities, which warrant further exploration.[8] Here, we found the whole plant extract inhibit the growth of *S. mutans*, *S. pyogenes*, *V. cholerae,* and *S. flexneri*.

Conclusion

Since it is an important herbal remedy in traditional medicine, *J. betonica* has been extensively investigated by various researchers for its phytochemical, pharmacological, and microbiological attributes. The plant extracts have great potential as antimicrobial compounds against microorganisms [Figure 1]. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The present study concluded that the selected medicinal plant has good activity against the clinically isolated pathogenic bacterial strains; further study is needed on the selected medicinal plant.

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