



EVALUATION OF ANTIBACTERIAL PROPERTIES OF JUSTICIA ADHATODA LEAF EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

Justicia adhatoda which belongs to the family Acanthaceae is a traditional medicinal plant widely used in Ayurvedic medicine. *Justicia adhatoda* is a multipurpose medicinal plant with several pharmacological properties like anti-inflammatory, anti-microbial, antioxidant, and also used to treat various diseases since ancient times to treat respiratory infections. *Staphylococcus aureus* is a major bacterial pathogen. The present study evaluates the antibacterial activity of different solvent extracts of *Justicia adhatoda* against *Staphylococcus aureus* (Gram Positive bacteria). The antibacterial activity of four solvent extracts of *Justicia adhatoda* was evaluated in vitro, with strains of the bacteria *Staphylococcus aureus*. The result of the present study supports the traditional use of studied medicinal plants in the treatment of bacterial infections and it also provides an important basis for the use of leaf extracts of *Justicia adhatoda* used to control infectious diseases caused by *Staphylococcus aureus* (Gram Positive bacteria). *Justicia adhatoda* leaf extract could be a green, eco-friendly alternative to synthetic chemical base hazardous artificial antibacterial agents through more research and systematic clinical trials.

KEYWORDS: *Justicia adhatoda*, Antibacterial activities, Plant extract *Staphylococcus aureus*

INTRODUCTION

Plants have been a crucial and traditional source of medicine for treating various diseases for thousands of years, as evidenced by research studies and historical practices. Various plant components contain phytochemicals, such as flavonoids, alkaloids, phenols, saponins, and tannins, which are important sources of bioactive substances with medicinal qualities (Patel S.B et al., 2021). These natural compounds have been utilized for their anti-microbial, anti-inflammatory, anti-diabetic, and other beneficial effects, contributing to the development of new drugs and treatments (Jawalkar Nilesh et al., 2020). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases (Chowdhury et al., 2009). *Adhatoda* species an important Indian medicinal plant has long been used in the Ayurvedic system of medicine (Ahmad S. et al., 2009, Selvarani, M., & Jeyasinga, V. 2020). Many plants have been used for antioxidant and antimicrobial properties which are due to secondary plant metabolites (Balandrin et al., 1985). Due to the emergence of antibiotic resistant pathogens, medicinal plants have found a better platform and could be an excellent alternate to combat the spread of multidrug-resistant (bacteria) microorganisms. Plant-based antimicrobials represent a vast untapped source for medicines with enormous therapeutic potential (Iwu M.W et al., 1999). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicrobials.

MATERIAL & METHODS

- 1. Collection and Identification of the Plant Material:** Leaves of the plant *Justicia adhatoda* were collected from the local area of Dist. Nandurbar and identified (with accession number.) by Herbarium In-Charge, Dept. of Botany, Dr. BAMU Cha. Sambhajinagar and COI gene sequence-based biological species identification by Paul Herbert Centre for DNA Barcoding and Biodiversity Studies, Dr. BAMU, Cha. Sambhajinagar. The sample is analysed for chloroplast DNA, *rbcL* gene sequence based biological species identification with the available verified (published) global sequence database of BOLD System V 3.1. The reference DNA sequence we obtained from the sample matches (99.81%) with *Justicia adhatoda*. Hence, the reference sample, *Justicia adhatoda* was confirmed
- 2. Extraction from Plant Materials:** The leaves of *Justicia adhatoda* were washed with tap water and dried in shade, then in an oven at 45°C and powdered in a grinder. A powder of leaves was stored in the airtight bottle and it will be used for further study. Powder was packed in filter paper and extract was extracted in the Soxhlets apparatus in a 1:10 ratio, i.e., 20 g of powder in 200 ml of solvent. The extract of the plant leaves of *Justicia adhatoda* was extracted in methanol, ethanol, acetone and chloroform. After eight hours of continuous extraction, the final extract was collected and kept open to evaporate the solvent. The remaining extract as a stock solution was stored at 4°C in the refrigerator.

3. Source of Bacterial Strains: *Escherichia coli* (Gram negative bacteria) organisms form part of the normal microbial flora of the intestinal tract of animals and humans. These organisms can also be found in vegetation, water and soil. It is gram negative, non-capsulated, short bacilli. Pure bacterial culture of *Escherichia coli* (Gram negative bacteria) was obtained from the pathology department of Sidhivinayak Diagnostic Centre Srirampur and again confirmed by cultural characteristics gram staining method and biochemical reactions like oxidase, urease, MR, VP, nitrate, citrate, indole and fermentation of sugars tests. The *E.coli* (bacterial culture) was maintained in nutrient broth at 4 °C under laboratory conditions.

4. Culture Media: The *E.coli* (Bacterial Stock Culture) were maintained in the nutrient broth at 4 °C under laboratory conditions. Mueller Hinton was used for the disc diffusion method of antimicrobial susceptibility testing of bacteria.

5. Preparation of Disc: Whatman filter paper No. 1 was used for the preparation of the disc with the help of a mechanical punching machine (size 6 mm). Punched disc circular pieces were collected and sterilised by the autoclaving method. These discs were used for further antimicrobial assays.

6. Antimicrobial Assay using Agar Disc Diffusion Method: Methanol, ethanol, acetone and chloroform solvent extracts of *Justicia adhatoda* (Family-Acanthaceae) were used for the determination of antimicrobial activity. Stock culture was maintained at 4 °C in the nutrient broth. The antimicrobial assay experiment was carried out according to the method of Bauer *et al.*, (1966). Mueller Hinton was used for the disc diffusion method of antimicrobial susceptibility testing of bacteria. By using a micropipette, 200 micrograms of slandered cell suspensions were spared on a MH Agar plate. Extracts of plant leaves of *Justicia adhatoda* were dissolved in methanol, ethanol, chloroform and acetone 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. Negative controls were prepared by using the same solvents employed to dissolve the samples. The disc was dipped in the respective concentration for one hour, then taken on the MH agar plate against *Escherichia coli* with the help of sterile forceps in aseptic laboratory technique. Plates were taken into the incubator for incubation at 37 °C for 24 hours. After 24 hours of incubation, the results were recorded. The effects of plant extract were compared with the standard. The effects of plant extract were compared with those of ceftizoxime as standard drugs at a 1 mg/ml concentration. The result was obtained by measuring the zone diameter. The results obtained by measuring the diameter of the zone of inhibition in millimeters Inhibition zones were measured and compared with Ceftriaxone as a standard reference antibacterial agent. Inhibition was recorded by measuring the zone of inhibition in millimetres (mm).

OBSERVATIONS

Evaluation of antibacterial activity was measured as the diameter of the zones of inhibition against the tested microbes.

Result of Zone inhibition in Millimeter (mm)							
Plant extract (Leaf)	Solvent	Negative control	Positive control Ceftizoxime 1mg/ml	Different concentration of plant leaves extract of <i>Justicia adhatoda</i>			
				25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
<i>Justicia adhatoda</i>	Methanol	0	28	8	13	16	19
	Ethanol	0	24	6	11	15	16
	Acetone	0	26	7	10	12	15
	Chloroform	0	25	7	12	14	15

*The Values are the average of three experimental results.

Table 1: Antimicrobial activity of different plant leaves extract of *Justicia adhatoda* (Family-Acanthaceae) against *Staphylococcus aureus*.

RESULTS

The clinical pathogen *Escherichia coli* (Gram Negative bacteria) was used to test the antibacterial activity of plant leaves in various solvent extracts of *Justicia adhatoda* (Family-Acanthaceae). Plant extract was prepared in different amounts using solvents such as methanol, ethanol, acetone, and chloroform, and the antibacterial activity was assessed using the disc diffusion method. Significant antimicrobial activity was recorded in the test samples. The present study showed higher antimicrobial activity in the order of methanol > ethanol > chloroform > acetone. The results of this study indicated that the plant leaf methanol extract of *Justicia adhatoda* exhibited higher antimicrobial activity.

CONCLUSION

Justicia adhatoda leaf extracts demonstrate significant antibacterial efficacy against *Staphylococcus aureus*, with activity influenced by extraction solvents and concentrations. As per experimental observations and results, *Justicia adhatoda* leaves extract could be green-eco-friendly, biodegradable alternative in prevention and treatment of bacterial infections.

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