In Vitro Evaluation of Antimicrobial Activity of *Justicia Adhatoda* Aqueous Leaf Extract

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ABSTRACT

Justicia adhatoda is a well-known medicinal plant in Ayurvedic and Unani medicine. Cough, whooping cough, cold, and clinging phlegm in the mouth, throat, and chest have been traditionally treated using J. adhatoda leaves. The present study aimed to evaluate antimicrobial activity and Minimum Inhibitory Concnetrations of J. adhatoda leaf extract against Gram-positive and Gram-negative bacterial strains such as Staphylococcus aureus and Pseudomonas aeruginosa. Based on the data presented here in, the largest zone of inhibition was found to be against P. aeruginosa (23mm) and the lowest inhibition zone was observed for S. aureus (13mm) at 100 µl/ml. The MIC values recorded for P. aeruginosa (6.25 µl/ml) and S. aureus was 12.5 µl/ml. As a result, the current method may be effective in identifying a new bioactive compound for the development of novel medications. Thus, it may be used as a strong antimicrobial agent against P. aeruginosa pathogens.

Keywords: *Pseudomonas aeruginosa, Staphylococcus aureus, Justicia adhatoda*

1. Introduction

The global prevalence of infectious diseases caused by bacteria is a major public health problem [1-2]. The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections [3]. The recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents [4] and are prompting a revival in research on the antimicrobial role of plants against resistant strains due to comparable safety and efficacy [5].

Plants are the prospective source of antimicrobial agents in different countries [6]. About 60 to 90% of the population in developing countries uses plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases [7]. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found to have antimicrobial properties [8]. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses [9].

In India, *Justicia adhatoda* is familiar as a medicinal herb finding its major place in various medical practice types such as Siddha, Ayurveda, Unani, homeopathy, and also other

ancient medicine [10]. Adhatoda parts, i.e., leaf, root, stem, and flower, are employed in many medicinal drug preparations. Especially the leaves are used in herbal remedies for diseases like chronic bronchitis, whooping cough, fever, cold, cough, jaundice, and asthma as a sedative expectorant, diarrhea, dysentery, and painful rheumatic inflammatory swellings.

Thus the present study investigated the activity of *Justicia adhatoda* leaf extract against human pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

2. Materials and Methods

The leaves of *Justicia adhatoda* were collected from Holy Cross College, Nagercoil (8.1560° N, 77.4151° E) and were brought to the laboratory. Leaves were washed three times with double distilled water and air-dried for 20 days. The dried leaves were ground to get a fine powder and stored in airtight container for further analyses.

Plant Collection and Preparation of the plant extract: 10 gm of the dried leaf sample was weighed, added to 100 ml of distilled water, and boiled at 80°C for 60 min. The extract was collected by simple filtration using Whatman no. 1 filter paper, and the extract was stored in a refrigerator at 4°C.

Antibacterial Activity: *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) were used for examining the bactericidal activity of plant extract. The inoculum was prepared aseptically by adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tubes, and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5 × 108 cfu/ml. Nutrient agar served as a medium for bacterial growth. Plant extract at concentrations of 25 µl, 50 µl, 75 µl, and 100 µl was tested against bacterial strains and compared with Streptomycin as a positive control. The plates were incubated at 37°C for 24 h, and the zone of incubation around the disc was observed. Three replicates were carried out for each extract against each of the test organisms. Data were expressed as mean ± standard deviation.

Determination of the Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used to determine the MIC according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The tested extracts were dissolved in 10% DMSO and diluted to a higher concentration. Then, serial ½ dilutions of extracts were prepared directly in a microtiter plate containing Mueller Hinton broth to obtain concentrations from 0.125 to 100 mg/mL. The bacterial inoculum was added to give a final concentration of 5×10^5 CFU/mL in each well. The positive control was used containing Streptomycin as the standard drug at final concentrations from 0.125 to 100 µl/ ml. The plate was covered with a sterile sealer and incubated for 24 h at 37°C.

Statistical analysis

The inhibition zones were calculated as means ± SD. The significance was evaluated by analysis of variance (ANOVA) using Microsoft Excel program. Significant differences in the data were established at the 5% level of significance. *P* values less than 0.05 were considered statistically significant.

3. Results

Antibacterial activity

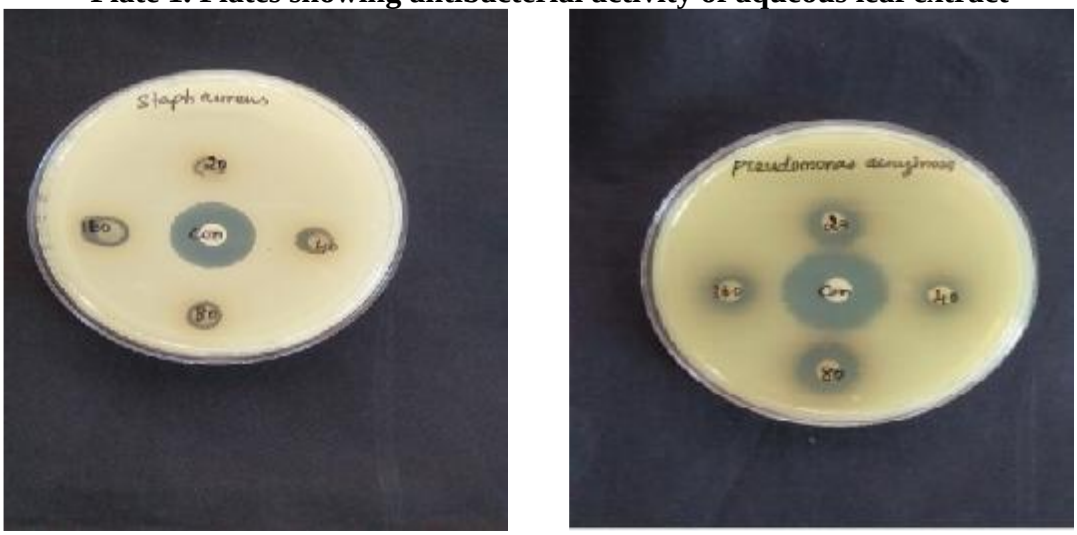
Evaluation of the antibacterial activity of aqueous leaf extract of *J. adhatoda* plant was determined by the disc diffusion method against selected human pathogens bacteria such as *Pseudomonas aeruginosa, and Staphylococcus aureus*.

Table 1. Antibacterial activities of aqueous leaf extract of *J. adhatoda* and standard drug

Concentration	*S. aureus*	*P. aeruginosa*	Streptomycin
25 µl	6±0.0	10±0.2	12±0.2
50 µl	9±0.1	15±0.5	18±0.6
75 µl	11±0.2	17±0.5	21±0.9
100 µl	13±0.3	23±1.0	26±1.9

The results showed better growth of inhibition against the tested bacterial strains based on the gradual increase of concentrations of 25 µl, 50µl, 75 µl and 100 µl extracts compared with the control. The diameters of inhibition zones are shown in Table 1 and Plate 1. The zone of inhibition formed by the leaf extracts was compared with that of the standard antibiotic Streptomycin. The aqueous leaf extract of *J. adhatoda* presented strong activity against *P. aeruginosa* with a diameter of inhibition zone of 23.0 mm and low activity against *S. aureus* (13.0 mm diameter) at a concentration of 100 µl. As the concentration of extracts was reduced the antibacterial activity was equally reduced. The inhibition zones of leaf extract obtained in this study indicated that leaf extracts have the efficiency to kill both Gram-negative and Gram-positive bacteria. There is no significant different between the means of the *P. aeruginosa* and *S. aureus* (p value - 0.059 < 0.05).

Plate 1. Plates showing antibacterial activity of aqueous leaf extract



Staphylococcus aureus ***Pseudomonas aeruginosa***

Minimum Inhibitory Concentrations

The effectiveness of the extracts on tested bacterial strains was determined by measuring the minimum inhibitory concentration (MIC). The samples of *J. adhatoda* leaf extract against Gram-positive and Gram-negative bacteria were tested for MIC using different concentrations from 0.125 to 100 µl/ml.

Table 2. Minimum inhibitory concentration (MIC) of aqueous leaf extracts of *J. adhatoda*

Bacterial Strains	MIC values
S. aureus	12.5 µl
P. aeruginosa	6.25 µl

The MIC value of *J. adhatoda* leaf extracts is shown in Table 4.2. *J. adhatoda* leaf extract showed the lowest MIC values against *P. aeruginosa* (6.25 µl/ml) followed by *S. aureus* (12.5 µl/ml).

4. Discussion

The MIC was considered the lowest concentration of the extract that completely inhibits bacterial growth. The lower the MIC the higher is the activity of the extract. Medicine plants contain a wide range of elements that can be used to treat chronic as well as infectious diseases. The most important advantage of herbal medicine is the relatively low cost compared to synthetic medicines as well as low-level side effects [11]. Antimicrobial resistance has been a major concern in the healthcare system globally [12]. Moreover, the wide usage of antibiotics

in the treatment of infectious diseases, and multiple drug resistance against pathogenic microorganisms have developed in recent years [13 -16]. Therefore, there is a need to develop alternative antimicrobial drugs to treat infectious diseases from various sources including plants [17-18]. The use of *J. adhatoda* plant extracts to test for antimicrobial activity has been brought forward as one of the ways of achieving this goal. This study evaluated the use of aqueous leaf extracts in treating selected bacterial pathogens (*S. aureus*, and *P. aeruginosa*).

The antimicrobial activity of leaf extract at different concentrations (25, 50, 75 and 100 µl/ml) was dictated by the zone of inhibition against the development of microorganisms. The most elevated antibacterial action was seen against *P. aeruginosa* (23.00±1.15) and afterward *S. aureus* (13.00 ± 0.64). It has been reported previously that the water extract of different plants usually yields significantly higher amounts compared to ethanolic extracts of the same plants [19]. Liu *et al.* [20] demonstrated that the antimicrobial properties of thyme are owing to its content of thymol that could bind to membrane proteins by hydrophobic bonding and hydrogen bonding, and thus changing the permeability of the membranes.

The antibacterial activity of the produced leaf extract against Gram-negative bacteria was higher than that against Gram-positive bacteria, which could have been possible due to the difference in the thickness of the peptidoglycan layer of their cell wall [21]. These activity variations can be attributed to the different properties that each bacterial strain constitutes its mechanism of inhibition [20]. Particularly, the cell wall composition of Gram-positive bacteria is seen to possess a thick layer of polysaccharide which is hard to be penetrated by leaf extracts, while the opposite is true with Gram-negative bacteria [22].

Mostafa *et al*. [23] that the difference in MIC of plant extracts is due to variations in their chemical constituents and the volatile nature of their components. The data revealed variability in the MIC of *J. adhatoda* extract, with the lowest MIC values of *P. aeruginosa* (6.25 µl/ml) and highest MIC values for S. aureus (12.5 µl/ml). Moreover, it has been reported that a large number of different chemical compounds such as (phenolic compounds and their derivative compounds, the esters of weak acid, fatty acid, terpenes, and others) are presented in ethanolic extracts of spice, and thus these chemical components can affect multiple target sites against the bacterial cells [24-25]. These findings indicate that the plant extracts tested in this study could be used to naturally eliminate or control the growth of pathogenic microorganisms.

5. Conclusion

It was concluded that *J. adhatoda* showed potential antibacterial activity against human pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Further phytochemical analysis of these plants will be helpful for the elucidation of lead molecules.

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