

Short Research Article

Evaluation of Antibacterial Activity of Endemic *Jeffreycazeylanica* Plant Found in Sri Lanka.

ABSTRACT

Aims: Determination of antibacterial efficacy of aqueous, methanol, dichloromethane, and hexane extracts from *Jeffreycazeylanica* leaves against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Methodology: Completely ballooned leaves of *J. zeylanica* were gathered, air-dried, and milled into fine powder. Then macerated in all four selected solvents for about 1-2 weeks, the extracts were obtained by vacuum evaporation under reduced pressure. Antibacterial activity was performed against *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) using the agar well diffusion method and disc diffusion method. The positive control utilized was Gentamicin. The inhibitory zone's diameter (in mm) was measured and noted. The entire experiment was done in triplicates.

Results: Findings from the study indicated that methanolic leaves extract (EC₅₀ 39.03mg/mL) had the highest effectiveness and potency against *S. aureus* using agar well diffusion method and, methanolic leaves extract (EC₅₀ 2.301mg/mL) had the highest effectiveness and potency against *S. aureus* using agar disc diffusion method.

Conclusion: This study designates that, leaves of *J. zeylanica* have potential antibacterial activity using aqueous, methanol, Dichloromethane(DCM), and hexane extracts. Among them methanolic extract showed the highest activity indicating the highest inhibition zones and the most susceptible organism is *S. aureus*. Additional study is required to understand the mechanism and active ingredients behind the antibacterial activity of further plant sections.

Keywords: Anti-bacterial activity, Agar Well Diffusion, Agar Disc Diffusion, *Jeffreycazeylanica*, Endemic plants, Traditional medicine, Sri Lanka

1. INTRODUCTION

Bacteria are found everywhere. They play an important role in maintaining the environment in which we live. Only a small percentage of bacteria in the world cause infections and diseases. Today, however, bacterial infections are having a major impact on the public health sector. Recently, infectious diseases accounted for one-third of all deaths in the world. WHO estimates that about 5,000 people die daily from infectious diseases worldwide [1]. Antibacterial agents are essentially important in reducing the global burden of infection and diseases. Microorganisms have acquired resistance to commonly used antibiotics due to the increased use of antibiotics. Bacterial infection is a proliferation of bacteria on or inside the body. Antibacterial activity destroys bacteria or suppresses their growth or their ability to reproduce. In novel medicine synthetic antibiotics are used to treat bacterial infections. Bacterial resistance to antibacterial is a rapidly growing problem with potentially devastating consequences. The availability and affordability of many antibiotics currently provided have been further impaired by the increasing number of Multi-

Drug Resistant (MDR) bacteria globally [2]. It consequently decreases the efficacy of the treatment regimens and raises morbidity, mortality, and healthcare expenditure levels [3].

The search for novel antibacterial drugs derived from plants has become increasingly important in recent years [4,5,6,7,8,9,10,37,38]. Plants have been used for thousands of years to treat a variety of ailments and are also a rich source of medicines. Medicinal plants can be defined as plant species with one or more bioactive molecules that are used to treat disease in folk medicine. Medicinal plants have great potential for discovering new bioactive compounds to combat resistant microorganisms. [11]. Traditional medicine (TM) is generally considered more accessible, economical, and accepted by local populations, so it can be used to achieve universal health coverage [12]. The WHO says 80% of developing countries still benefit from using traditional herbs derived from medicinal plants. Sri Lanka's rich and diverse flora means that indigenous peoples have accumulated traditional remedies for various ailments. [13,14]. Because of its tropical nature, Sri Lanka has the ideal climate for evergreen plants as compared to other countries, and as a result, the Sri Lankan Flora is rich in medicinal plants.

J. zeylanica is an herbaceous plant that is native to Sri Lanka. It displays a range of ethnomedical characteristics. It belongs to the ASTERACEAE family. *J. zeylanica* is a small undershrub that grows to be between 0.5 and 1 m tall and has numerous divaricate, cylindrical branches that, when young, are delicately tomentose. The native distribution of *Jeffreyzia zeylanica*, an endemic to Sri Lanka, is in the dry-wet zone. Common in dry zone home gardens and Chena cultivation. It is commonly referred to as Pupula in Sinhala and Kuppilay in Tamil. It has a wide variety of ethnomedicinal traits. *Jeffreyzia zeylanica* is used in fractures as it promotes the fusion of bones, it is applied as an oil that is prepared by using the extractions of bark and leaves. The leaves are ground into a paste and applied to boils to encourage suppuration. Roasted with turmeric, it helps with eczema on the feet. Internally, the leaf extract is used to treat asthma. It is also useful as an emetic, especially in cases of food poisoning, diarrhea, and dysentery. This plant can be used for wounds and abscesses and as an anti-venom agent [15,16,36].

Steroids, triterpenoids, alkaloids, phenolic chemicals, and flavonoids have been reported to be found in the plant *J. zeylanica*, according to phytochemical investigations. There are several different secondary metabolites abundant in plants. Among them, tannins, alkaloids, phenolic compounds, and flavonoids have been found in vitro to have antibacterial potential [17,35]. Bacterial strains have been chosen to investigate anti-bacterial properties based on the plant's ethnomedical use. Because of the therapy for wounds and abscesses, Gram-Positive *Staphylococcus aureus* and Gram Negative *Pseudomonas spp.* were chosen. For diarrhea and dysentery, Gram Negative *E. coli* was chosen mainly. Because plants may contain antibacterial components, they can be used to evaluate medications to aid in the cure of diseases.

2. METHODOLOGY

2.1 Plant material collection, Identification, and Authentication

Matured, well-expanded leaves of *J. zeylanica* about 4 kg were collected from the Matara district in the southern province of Sri Lanka. They were collected during the flowering season. The plant was identified and authenticated at the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka.

2.2 Preparation of Aqueous, Methanol, Dichloromethane, and Hexane Extracts of *J. zeylanica* leaves.

Measured the raw weight of the plant leaves (excluding stems and barks). The leaves were separated from the stems and barks and washed comprehensively using tap water. Air-dried the leaves without exposing them to direct sunlight. Evaluated the dried weight of the leaves, until the weight reached a constant level. When the weight of the leaves reached a constant level, the leaves were ground into a fine powder using a grinder [18]. Calculate the total weight of the powdered material. The cold maceration process was used for the extraction of

Jeffreycazeylanica leaves. Polar and non-polar solvents were used for the maceration process. Solvents or menstruum were aqueous, methanol, dichloromethane (DCM), and hexane. Powdered plant material was placed in a closed container (amber-colored) and stored at room temperature for 1-2 weeks with frequent agitation until soluble matter is dissolved. [19]. A rotary vacuum evaporator was used to concentrate the filtrate. This instrument is to rotate the round bottom flask while lowering the pressure within to allow the solvent to evaporate more quickly below its boiling point. A solvent will evaporate by heating below the solvent's boiling point, and the dissolved compounds will eventually precipitate, protecting the solvent's constituents from harmful consequences caused by elevated temperatures. Plant extractions were collected into four sterile petri dishes and labeled and sealed the Petri dishes and stored in the freezing compartment.

2.3 Preparation of Microorganisms

American Type Culture Collection (ATCC) strains of bacteria were obtained from the Medical Research Institute (MRI), Colombo 08, Sri Lanka. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were acquired using Nutrient agar (NA) slants. Stored at 4 °C in the refrigerator until subcultured. The bacterial stock cultures were stored at 4 °C and were sub-cultured on freshly prepared and quality-controlled NA plates. Plates were incubated at 37°C for 18-24 hours. After incubation, the plates were stored in the refrigerator until further use. For the experimental procedure stock culture plates were taken before the experiment and bacterial strains were again sub-cultured on freshly prepared sterile NA plates to get the fresh organism culture colonies [20].

2.4 Preparation of Standard Concentration and Positive Control for Agar Well Diffusion Method,

For the preparation of the standard concentrations of the different concentrated plant materials, Dimethyl sulfoxide (DMSO) was used for the resuspension of the plant extracts, the used concentrations were 400 mg/mL, 300 mg/mL, 200 mg/mL, 100 mg/mL, 75 mg/mL, 50 mg/mL, 20 mg/mL and 10 mg/mL. A 400 mg/mL concentration was prepared by measuring 1.6 g from the concentrated plant extract and dissolving it in 4 mL of DMSO. It was used as the standard stock and other concentrations were derived from the prepared stock using distilled water dilutions [21].

Commercially available IV Gentamicin 80 mg/2mL (40 mg/mL) vial was used as the positive control [22].

2.5 Anti-bacterial Activity Screening Agar Well Diffusion Method

Antibacterial activity was performed against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Standard bacterial suspensions were prepared according to the standard guidelines. 0.5 MacFarland standard used when preparing the bacterial suspensions. Aseptic conditions were followed during the experimental study. MHA plates were prepared for the study. Wells were cut within the agar media using a sterile pipette tip (diameter-6 mm). A maximum of 5 wells were prepared in a 90 mm Petri dish. The bottom of the well was sealed using sterile MHA media of 10 µL. After the solidification of the sealing media, 100 µL of the prepared standard concentrations of the plant extract were added to each well along with the positive and negative controls. Gentamicin was used as the positive control while aqueous, methanol, DCM, and hexane were used as the negative control for appropriate plant extract. The solvent of extraction was used as the negative control to identify any interference for the zone of inhibition. Plates were incubated at 37 °C for 18-24 hrs. The diameter of inhibition zones of the bacterial strains was measured and reported in mm, the whole procedure was done in triplicates [23,24].

2.6 Preparation of Antibiotic Discs, Standard Concentration, and Positive Control for Agar Disc Diffusion Method,

Whatman (no 1) filter papers were used to prepare the antibacterial discs. The filter paper was punched to make the discs obtain a 6 mm diameter. Then the discs were sterilized using the autoclave. Discs were prepared by impregnating 5 µL of the plant extract standard concentration.

Similarly negative control was prepared by impregnating 5 μ L of the relevant solvent. In this experimental study, the impregnating volume for the disc was chosen by inserting different volumes into the prepared discs[25].

Plant extracts with standard concentrations were made as same as the agar well diffusion method. The used concentrations were 400 mg/mL, 300 mg/mL, 200 mg/mL, and 100 mg/mL.

For the positive control, a standard Gentamicin antibacterial disk was used (Oxoid™, 10 μ g).

2.7 Anti-bacterial Activity Screening Agar Disc Diffusion Method

The antibacterial effect was screened using the disc diffusion method according to the standard guidelines. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used as the test organisms equally to the agar well diffusion method. The test was done using sterile MHA plates. Five discs were placed in one MHA plate, along with the positive and negative control. The standard Gentamicin disc was used as the positive control, and the solvent used for the extraction was used as the negative control. Control organisms were inoculated onto the MHA plate using a cotton swab, and standard organism suspension was prepared, using the 0.5 McFarland standard. After placing the prepared discs, concerning aseptic conditions plates were incubated at 37 °C for 18-24 hours inside the incubator. The plates were examined the next day. The diameter of inhibition zones of the bacterial strains was measured and reported in mm, the whole procedure was done in triplicates.

3. RESULTS AND DISCUSSION

J. zeylanica is an endemic plant in Sri Lanka, so there was very limited literature regarding its antibacterial activity[26,27,28]. According to previous literature, it has antibacterial potential. In this study, we used aqueous methanol, DCM and hexane leaf extracts of *J. zeylanica*. These solvents were selected according to their polarity [29]. Several solvents with differing polarities must be employed to accurately extract diverse secondary metabolites from plants. The polarity from least polar to most polar, hexane < dichloromethane < methanol < aqueous [30]. The obtained results were analyzed using GraphPad Prism 8 (version 8.2.1).

3.1 Well Diffusion Method Results:

According to the observed results. The maximum inhibitory zone was observed in methanolic leaf extract of *J. zeylanica*, against *S. aureus* 29.33 \pm 0.33 mm (Mean \pm SEM). In compliance with the revealed data compared to other extracts (aqueous, DCM, Hexane), methanolic leaf extract has remarkable antibacterial activity against *S. aureus*. The maximum inhibitory zone was observed in aqueous leaf extract against *P. aeruginosa* 24 \pm 0.57 mm (Mean \pm SEM) in contrast with other extracts methanolic extract was most effective and had the higher antibacterial potential. The maximum inhibitory zone was observed in methanolic leaf extract against *E. coli* 12.66 \pm 0.33 mm (Mean \pm SEM), methanolic extract had the highest antibacterial potency.

When comparing the EC₅₀(mg/mL) values, the following information was found regarding the antibacterial activity of the agar well diffusion method. According to Table 1. The antibacterial activity of leaf extract against *S. aureus* was methanol (39.03) > DCM (124.5) > aqueous (193.3) > hexane (312.0). The antibacterial activity of leaf extract against *P. aeruginosa* was aqueous (183.0) > methanol (1392.0). The antibacterial activity of leaf extract against *E. coli* was methanol (39.01) > aqueous (177.1).

To Sum up the agar well diffusion method that we had done, the methanolic extract is the most efficient against all three selected organisms, but it was remarkable for Gram-positive *S. aureus* (ATCC 25923).

Table 1. EC₅₀ (mg/mL) of aqueous methanol DCM and hexane extracts of *J. zeylanica* against *S. aureus*, *P. aeruginosa*, and *E. coli*. Using the agar well diffusion method

Microorganism	EC ₅₀ (mg/mL)			
	Aqueous	methanol	DCM	Hexane
<i>S. aureus</i>	193.3	39.03	124.5	312
<i>P. aeruginosa</i>	183	1392		
<i>E.coli</i>	177.1	39.01		



Fig1. Inhibition zones against *S. aureus* in methanolic leaf extract of *J. zeylanica*

3.2 Disc Diffusion Method Results:

The maximum zone of inhibition against *S. aureus* was observed in DCM 14.66 ± 0.33 mm (Mean \pm SEM) and methanol 14.33 ± 0.33 mm (Mean \pm SEM). Methanolic leaf extract had the most effective antibacterial activity against *S. aureus*. The maximum inhibitory zone against *P. aeruginosa* was observed in methanolic leaf extract 12.33 ± 0.33 mm (Mean \pm SEM). Methanolic leaf extract has the most effective antibacterial activity against *P. aeruginosa*. The maximum zone of inhibition against *E. coli* was observed in methanolic leaf extract 11 ± 0.57 mm (Mean \pm SEM). Methanolic leaf extract was the most effective against *E. coli*.

The following information was found when comparing EC₅₀ (mg/mL) values using the disc diffusion method. According to Table 2. The antibacterial activity of leaf extracts against *S. aureus* using the agar disc diffusion method was methanol (200) > hexane (287) > DCM (251.1). The antibacterial activity of leaf extract against *P. aeruginosa* was, methanol (5.239) > hexane (270.2) > DCM (296.8). The antibacterial activity of leaf extract against *E. coli* was methanol (233) > hexane (272.4).

When narrowing down the overall antibacterial activity of *J. zeylanica* leaves extracts has been done using the disc diffusion method, methanolic extract was the best, it is effective against all three selected organisms.

Table 2. EC₅₀ (mg/mL) of aqueous methanol DCM and hexane extracts of *J. zeylanica* against *S. aureus*, *P. aeruginosa*, and *E. coli*. Using the agar disc diffusion method

Microorganism	EC ₅₀ (mg/mL)			
	Aqueous	methanol	DCM	Hexane
<i>S. aureus</i>		200	287	215.1
<i>P. aeruginosa</i>		5. 329	296.8	270.2
<i>E. coli</i>		233		272.4



Fig2. Inhibition zones against *S. aureus* in methanolic leaf extract of *J. zeylanica*

The global burden of antibiotic resistance is a major concern in worldwide healthcare settings. As an alternative, using plant extracts to combat developing antimicrobial resistance will be efficient and economical [31]. *Jeffreyia zeylanica* is an endemic plant in Sri Lanka with significant ethnomedical value. In this study, the antibacterial of *J. zeylanica* is evaluated. According to the study, it evidenced the antibacterial potency of the plant *J. zeylanica*. All four solvent extracts have shown appreciable anti-bacterial activity, but the methanolic leaf extract of *J. zeylanica* showed remarkable results. Methanol is a polar compound, we used methanol to extract polar secondary metabolites from leaves of *J. zeylanica*. In compliance with the results plant's polar secondary metabolites are more contributed to antibacterial activity. Literature suggests that *J. zeylanica* contains phenolic compounds, flavonoids, and saponins. These are more polar secondary metabolites in the plant and phytochemical studies done on them revealed that they were responsible for antibacterial activity [32,33]. *Staphylococcus aureus* is the most susceptible organism. *S. aureus* is Gram Positive they are more susceptible to antibiotics than Gram Negative organisms do their cell wall structure [34].

4. CONCLUSION

In conclusion, this study indicates the potent *in vitro* antibacterial activity of aqueous, methanol, dichloromethane, and hexane extracts of leaves of *J. zeylanica* against *S. aureus*, *P. aeruginosa*, and *E. coli*. The methanolic leaf extract showed remarkable results against *S. aureus*. Further studies need to be performed to determine the mechanism of antibacterial activity and active components of *J. zeylanica*. Growing interest in traditional folk medicine could lead to the discovery of new therapeutic agents, since *J. zeylanica* contains antibacterial potency and is useful for the development of pharmaceutical drugs, it will be more economical and more accessible.

7. REFERENCES

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