



Research Article

STUDIES ON ANTIMICROBIAL ACTIVITY OF *ZIZIPHUS MAURITIANA* LAM.

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ABSTRACT

Ziziphus mauritiana Lam. is a medium sized tree (Rhamnaceae) grows in almost all parts of the country. It is a potent medicinal plant with many biologically active components such as phenolics, flavonoids, triterpenic acids, polysaccharides and saponins. It majorly possesses anti cancer, anti oxidant, wound healing, antifertility, anti inflammatory, antiulcer, antidiarrhoeal and antifungal activities. In the present study, anti microbial activity of *Ziziphus mauritiana* was tested with ethanol and methanol extracts against various medically important bacteria such as *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and pathogenic fungi such as *Candida albicans* and *Trichoderma viride*. The invitro anti microbial activity was performed by agar well diffusion method. Of the two leaf extracts, ethanol extract from *Z. mauritiana* leaves exhibited significant antibacterial and antifungal activity. In anti bacterial studies, the results revealed that significant zone of inhibition was observed in ethanol extract of *Z. mauritiana* leaf against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. In anti fungal studies, ethanol leaf extract showed promising results against *Candida albicans* and *Trichoderma viride*.

KEYWORDS: *Ziziphus mauritiana*, ethanol extract, antimicrobial, Well diffusion method.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plant antimicrobials offer potentially new classes of agents as they can substitute for synthetic antibiotics and drugs^[1]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to plant extracts and biologically active compounds isolated from plant species in herbal medicines^[2]. Many of the plant products have important therapeutic agents, which are represented by the various phytochemicals like alkaloids, glucosides, flavanoids, mucilages, enzymes etc. Even now, drugs from higher plants continue to occupy an important niche in modern medicine.

Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated^[3]. One such medicinal plant is *Ziziphus mauritiana* Lam., a member of the family Rhamnaceae commonly known as ber and is mostly found almost in all parts of the country.

Ziziphus mauritiana Lam. is a low branched deciduous tree with spreading crown, dark greenish black bark having irregular crack and strong reddish hardwood with oblong and elliptic leaves. It is known as Ber (Hindi), Indian jujube (English) and Badarh (Sanskrit). It is found throughout India, in dry

deciduous forests up to 1500 m. In traditional medicine of Ayurveda, unripe fruits are used to pacify "Vata", the leaves, fruits, bark & even roots are used to treat a variety of ailments including cold, flu and malnutrition related diseases in children, convulsions and indigestion^[4]. The leaves are applied as poultices and are helpful in liver troubles, asthma, fever and to treat sores^[5] and the roots are used to cure and prevent skin diseases^[6]. All the parts of this plant are very effective against different types of diseases. Its leaves are useful in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea. The leaves of *Z.mauritiana* are also used in the treatment of liver diseases, asthma and fever^[7]. The fruit has been used as anodyne, sedative, tonic anticancer and potent wound healer ^[8]. The fruit^[9], leaves^[10] and seed^[11] extracts have been shown to exhibit antioxidant activity, where as bark ^[12] is reported to have cytotoxicity against different cancer cell lines.

Considering the vast potentiality of *Ziziphus mauritiana* as sources for antimicrobial drugs, this study was undertaken to identify the antibacterial and antifungal activity of *Z.mauritiana* leaf.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Z.mauritiana* free from disease were collected from Muhavoor, near Rajapalayam and the plant (Plate -1) was identified and authenticated by Dr. M. Palanisamy, Botanical survey of India, Southern regional centre, Coimbatore.

Preparation of plant extract

Thoroughly washed dried leaves of *Ziziphus mauritiana* were dried in shade for five days and then powdered with the help of Warring blender. Leaf extract was prepared separately and successively using Soxhlet apparatus with different solvents namely ethanol and methanol. The extract preparation was performed at Govt. Arts College, Coimbatore. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies

The screening of anti microbial activity of leaf extracts of *Z.mauritiana* were carried out on actively grown cultures of bacteria such as *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and in fungal cultures of *Candida albicans* and *Trichoderma viride*. All the strains were procured from Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore. The bacterial cultures were maintained on nutrient agar medium at 37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

Preparation of Inoculum

The selected gram positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C. It was then centrifuged at 10,000 rpm for 5 min and the pellet was

suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculum (*Candida albicans* and *Trichoderma viride*) were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

Antimicrobial Screening

The leaf extracts of *Z.mauritiana* were tested for antimicrobial activity against the selected bacteria and fungi. Nutrient agar media were used for bacterial growth and Potato dextrose agar media were used for fungal culture. The media were prepared and then autoclaved at 121°C for 15 minutes and were poured on petriplates and allowed to solidify.

Well Diffusion Method

Well diffusion method is used to evaluate both antibacterial and antifungal activity^[13]. The prepared culture plates were inoculated with selected strains of bacteria and fungus using spread plate method. The wells were made on the agar surface with sterile cork borer. The extracts were poured into the well using micropipette with the concentration of 20, 40 and 60 µl/ml. Streptomycin was used as positive reference standard to determine the sensitivity of each microbial species tested. The bacterial culture plates were incubated at 37°C for 24 hours and fungal culture plates were incubated at room temperature for 1 week. The zone of inhibition was calculated by measuring the diameter of the zone around the well in millimeters (mm).

RESULTS AND DISCUSSION

The results obtained for anti microbial studies revealed that *Z.mauritiana* leaf extract possess potential anti bacterial activity and antifungal activity and the data was presented in Table 1 and 2.

Table 1: Antibacterial activity of leaf extracts of *Ziziphus mauritiana* Lam.

| Test Organisms | Zone of inhibition (mm) | | | | | | Std. (Streptomycin) |
|-------------------------------|-------------------------|------------------|-----------------|------------------|-----------------|------------------|---------------------|
| | 20 µl/ml | | 40 µl/ml | | 60 µl/ml | | |
| | Ethanol extract | Methanol extract | Ethanol extract | Methanol extract | Ethanol extract | Methanol extract | |
| <i>Streptococcus pyogenes</i> | 10 | 08 | 10 | 09 | 14 | 14 | 11 |
| <i>Staphylococcus aureus</i> | 18 | 14 | 16 | 15 | 16 | 16 | 11 |
| <i>Escherichia coli</i> | 22 | 20 | 18 | 16 | 20 | 18 | 10 |
| <i>Klebsiella pneumoniae</i> | 19 | 15 | 16 | 16 | 18 | 18 | 10 |

Table 2: Antifungal activity of leaf extracts of *Ziziphus mauritiana* Lam.

| Test Organisms | Zone of inhibition (mm) | | | | | | Standard (Streptomycin) |
|---------------------------|-------------------------|------------------|-----------------|------------------|-----------------|------------------|-------------------------|
| | 20 µl/ml | | 40 µl/ml | | 60 µl/ml | | |
| | Ethanol extract | Methanol extract | Ethanol extract | Methanol extract | Ethanol extract | Methanol extract | |
| <i>Candida albicans</i> | 05 | 05 | 08 | 08 | 8.5 | 06 | 08 |
| <i>Trichoderma viride</i> | 05 | 05 | 06 | 06 | 09 | 08 | 08 |

In anti bacterial studies, 20 µl/ml of ethanol extract from *Z.mauritiana* leaf was found to be effective in inhibiting *E. Coli* (22mm), *Klebsiella pneumoniae* (19 mm) and *Staphylococcus aureus* (18 mm) except *Streptococcus pyogenes* which was restrained at 60 µl/ml of leaf extract (Graph -1), (Plate-2). Similar results were also observed in methanol extract from *Z.mauritiana* leaf (Plate -3).

From the table, it was evident that the plant behaved differently for different bacteria and altered their efficiency based on the solvent used. Of the two extracts studied, ethanolic leaf extract exhibited promising results than methanol leaf extracts.

A significant antifungal activity was seen in 60µl/ml of ethanol extract from *Z.mauritiana* leaf that was effective in controlling both *Candida albicans* (8.5 mm) and *Aspergillus fumigatus* (9mm) (Graph- 2) (Plate 4). Same results were also seen at 60µl/ml of methanol extract from *Z.mauritiana* leaf.

The result of present study showed that the ethanol extracts of *Ziziphus mauritiana* leaf exhibited potential antibacterial activity against the tested pathogens at 20 µl/ml concentration. In anti fungal activity, *Z.mauritiana* leaf extracts were found to be moderately active against the tested fungal organisms at high concentration, which may be due to the variation in the fungal and bacterial cell wall components.

Some of the organic compounds detected in this plant extract include tannins, saponins, poly phenols, glycosides etc. These compounds have variously been reported to have anti microbial activity and could be the reason for the activities recorded against these test organisms. Tannins have general anti microbial and anti oxidant activities^[14] and saponins possess anti cancer, anti fungal and anti inflammatory properties and it is considered to be a bioactive anti bacterial agent^[15,16]. Hence, this finding can form the basis for further studies to optimize the preparation of the herbal extract and to further evaluate them against a wider range of bacterial strains.

CONCLUSION

In the present study, it is found that *Z.mauritiana* leaf extracts possess significant antimicrobial activity against the tested microorganisms and the plant contains potential antimicrobial components for the therapy of microbial infections. Therefore the results justify the use of the leaf extract of *Z.mauritiana* in treating these pathogenic strains and these may serve as leads for the development of new pharmaceuticals. It is essential that the plant can be further explored for its phytochemical profile to identify the active constituents for drug discovery and development.

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Study Photographs

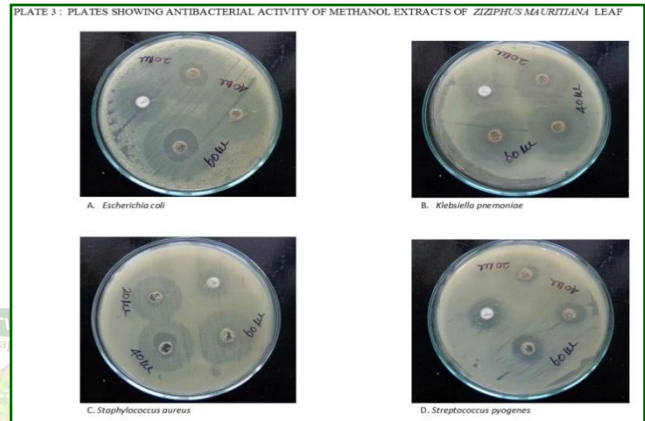
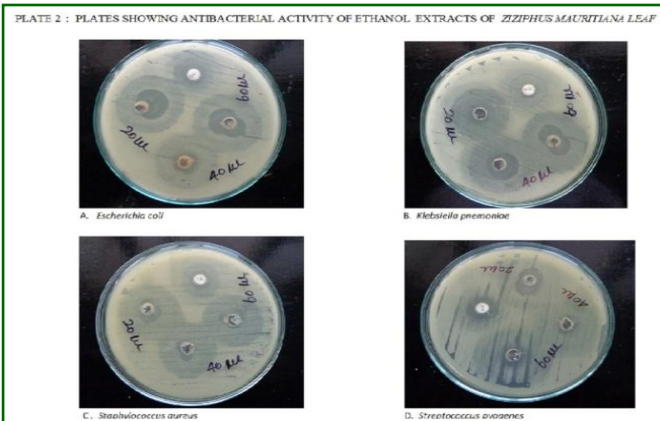


Plate 1: *Ziziphus Mauritiana* -Habit

