International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 11-11-2024, Accepted: 12-12-2024, Published: 27-12-2024
Volume 9, Issue 1, 2024, Page No. 58-60

Mechanisms of antifungal and antibacterial properties of *Azadirachta indica* leaf extract on *Aspergillus flavus* and *Xanthomonas citri* PV. *citri*

NT Pawar, BT Pawar, MT Kuchekar, RJ Sawant
Department of Botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra, India

Abstract
Azadirachta indica, commonly known as neem, is a tree native to the Indian subcontinent and has been an integral part of traditional medicine for centuries. Known for its broad-spectrum therapeutic properties, neem is widely recognized for its antimicrobial, anti-inflammatory, and antipyretic effects. This research aims to evaluate the antifungal and antibacterial activity of neem extracts, focusing on their potential as effective alternatives to synthetic drugs. By testing both aqueous and ethanol extracts of neem against a range of bacterial and fungal pathogens, this study seeks to assess the spectrum and potency of its antimicrobial effects. The antibacterial activities of medicinal plant extract were tested by the standarddisc diffusion method. The clinically pure identified three major pathogens like *Xanthomonas citri* pv. *citri* were taken for analysis.

Keywords: *Azadirachta indica*, *Xanthomonas citri*, antifungal and antibacterial

Introduction

Azadirachta indica, commonly known as neem, is a tree native to the Indian subcontinent and has been an integral part of traditional medicine for centuries. Known for its broad-spectrum therapeutic properties, neem is widely recognized for its antimicrobial, anti-inflammatory, and antipyretic effects. The bioactive compounds found in neem, such as azadirachtin, nimbin, and other triterpenoids, flavonoids, and alkaloids, are believed to contribute to its potent antibacterial and antifungal properties Tripathi, 2007 [7]. These compounds have made neem a subject of considerable research in the field of phytomedicine, particularly as a natural alternative to synthetic antibiotics and antifungal agents. The emergence of antimicrobial resistance (AMR) has become a global health threat, rendering many conventional antibiotics and antifungals ineffective against common pathogens. This situation has heightened the need for alternative antimicrobial substances, particularly those derived from plants. Neem has shown promising antimicrobial activity against a wide range of bacterial and fungal pathogens, including drug-resistant strains. Studies have reported its effectiveness against both Gram-positive bacteria (such as *Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (including *Escherichia coli* and *Pseudomonas aeruginosa*) Sharma 2012 [4]. Additionally, neem extracts have demonstrated antifungal activity against a variety of fungal species, including *Candida albicans*, *Aspergillusniger*, and Dermatophytes, which are responsible for infections of the skin, mucosa, and systemic fungal diseases Ghosh, 2010 [2], Shobha 2016 [5].

Neem's antimicrobial properties are attributed to its ability to inhibit microbial growth, disrupt cell wall integrity, and interfere with various cellular mechanisms, such as protein synthesis and enzyme activity. The use of neem in traditional medicine, particularly for treating skin infections, dental diseases, and gastrointestinal disorders, has spurred further investigation into its antimicrobial efficacy. Moreover, neem's relatively low toxicity and wide availability make it an attractive candidate for developing natural antimicrobial agents. This research aims to evaluate the antifungal and antibacterial activity of neem extracts, focusing on their potential as effective alternatives to synthetic drugs. By testing both aqueous and ethanol extracts of neem against a range of bacterial and fungal pathogens, this study seeks to assess the spectrum and potency of its antimicrobial effects. The findings of this study may not only support the use of neem in traditional medicine but also provide a scientific basis for its incorporation into modern therapeutic approaches, especially in the context of growing concerns over antimicrobial resistance. Furthermore, identifying the key bioactive compounds responsible for neem's antimicrobial activity could lead to the development of novel, plant-based pharmaceuticals with broad-spectrum efficacy.

Materials and methods

1. Preparation of aqueous extract:

The fresh, mature and infection free plants parts (root, stem bark, leaves,) were collected. The freshly collected plant parts were washed with fresh water followed by sterile distilled water and finally ground with sterile water at the rate of 1ml/g of tissue in a mortar and pestle. The macerate was succeeded through cotton wool to get the extract. The extract was succeeded through two layers of muslin cloth and finally through Whatman No. 1 filters paper. This formed the standard extract solution (100%). From this various concentration extract was prepared (Shekhawat and Prasad 1971) [6].

2. Preparation of Solvent Extracts:

Extractions were prepared in ethanol, acetone, water and methanol. Extraction was done at room temperature by simple extraction method (Lopez *et al.* 2008) [3]. Dried powder of plant parts (10 g) was mixed with 100 ml solvent (i.e. ethanol, acetone, water and methanol) in 250 ml conical flasks. The flasks were plugged tightly with cotton and wrapped with paper. All the conical flasks were kept on shaker for 24 h. Then it was allowed to stand for five hours for settling of the plant material. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 40-50 °C to

make the final volume one fifth of the original volume. It was stored at 4 °C in airtight bottles for further studies. These extracts were used for the screening of antifungal and antibacterial activity. For the determination of inhibitory concentration of extract to bacteria, plant extract were used. Plant extract residue were achieved by evaporating extracts using rotary evaporator at 50 °C.

3. Antifungal assay of Plant Extract:

The antifungal activities of medicinal plant part extract were tested by the standardagar well diffusion method. Identified major plant pathogens like*Aspergillusflavus*were takenfor analysis. Three plates for organism in such a way 09 plates containing solidified PDA (Potato Dextrose Agar) media were prepared. The disk diffusion method was used to check the antifungal activity of medicinal plant leaf extract.

4. Antibacterial assay of Plant Extract:

The antibacterial activities of medicinal plant extract were tested by the standarddisc diffusion method. The clinically pure identified three major pathogens like *Xanthomonas citri* pv. *citri* were taken for analysis. Three plates for organism in such a way 09 plates containing solidified nutrient agar and nutrient broth medium were prepared. Fresh autoclaved nutrient agar medium containing Beef extract-3gm, Glucose-2.5gm, Peptone-5gm, Agar-15gm, Distilled water 1000ml, and pH – 6.8. Nutrient broth Solution used for the preparation of bacteria suspension. The disc diffusion method was used to check the antibacterial activity of medicinal plant extract.

Result and discussion

A. Antifungal Activity of *Azadirachta indica* leaf Extract:

Different solvent extracts *viz* Acetone, Ethanol and aqueous medicinal plants *i.e*. *Azadirachta indica* were tested to find out their antifungal activity against *Aspergillus flavus*, and using the paper disc diffusion assay. The application of Leaf extract of *Azadirachta indica* showed significant effect against *Aspergillus flavus* with ethanol as well as acetone solvent. Table 1.1 showed the effects of Leaf extract of *Azadirachta indica*, against *Aspergillus flavus*. The acetone extract of *Azadirachta indica* showed maximum zone of inhibition against the tested pathogen at 20 mg/ml concentration. 40 mg/mlconcentration showed 15.8 mm zone of inhibition against the fungus. At 60 mg/mlconcentration the inhibition zone was determined to be as 17.9 mm which was lower than 25.8 mm inhibition zone at 80 mg/ml concentration. Following acetone extract maximum zone of inhibition observe in ethanol extract at 60 mg/ml concentration which is a bit lower than 80 mg/ml concentration which is clearly showed in Figure 1.1.

Table 1.1: *In-vitro* antifungal activity of Leaf extract at various concentrations against *Aspergillus flavus*

Plant Name	Concentration mg/ml	Zone of inhibition against *Aspergillus flavus* in mm		
		Ethanol	Acetone	Aqueous
Azadirachtaindica	20mg/ml	10.3	12.5	0.6
	40mg/ml	12.4	15.8	2.4
	60mg/ml	16.8	17.9	4.8
	80mg/ml	20.7	25.8	10.4

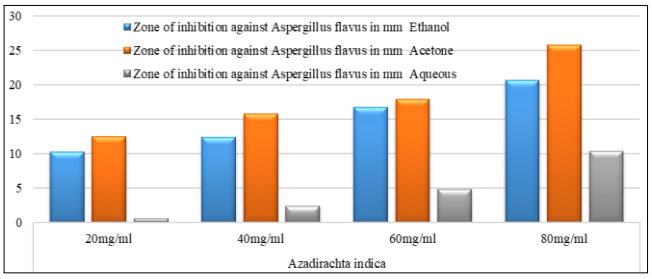

Figure 1.1: *In-vitro* antifungal activity of Leaf extract at various concentrations against *Aspergillus flavus*

B. Antibacterial Activity of *Azadirachta indica*

In this research work we also observed the antibacterial activity of methanolic extract aqueous and acetone extract of *A. indica* plant parts using agar well diffusion method, pathogenic bacteria *i.e*. *Xanthomonas citri* pv. *citri* at different concentration (40µg/ml, 80µg/ml, 120µg/ml, 160 µg/ml, 200µg/ml). After 24 hours of incubation we observed the different zone of inhibition on different concentration. The effect of leaf extract of *Azadirachtaindica* on the growth of *Xanthomonas citri* pv. *citri* was observed, the growth was noted in the form of diameter of colony growth in mm. The results are recorded in the table 1.2, and Figure 1.2. It was seen that, as the concentration of medicinal plants increases, the growth of bacrerial zone decreases with respect to methanol and acetone solvent. But the colony growth after treated with aqueous extract not that much effective. Data from table 1.2, and Figure 1.2 indicate the efficacy of leaf extract against *Xanthomonas citri* pv. *citri*. The leaf extract of *Azadirachta indica* with methanol and acetone was found to be significantly effective in inhibition the pathogen growth recording inhibition of diameter as 21.5 mm followed by acetone extract 18.2mm, at their highest concentration of plant extracts i.e. 200µg/ml. aqueous leaf extract of *Azadirachta indica* were the not effective treatment by recoding the inhibition diameter as 0.8.mm. as compared to other treatments.

Table 1.2: *In-vitro* antibacterial activity of Leaf extract at various concentrations against *Xanthomonas citri* pv. *Citri*

Plant Name	Concentration µg/ml	Zone of inhibition against *Xanthomonas citri* pv. *citri* in mm		
		Methanol	Acetone	Aqueous
Azadirachtaindica	40µg/ml	4.1	3.7	0.0
	80µg/ml	7.2	5.4	0.0
	120µg/ml	14.6	9.6	0.2
	160µg/ml	18.6	15.3	0.5
	200µg/ml	21.5	18.2	0.8

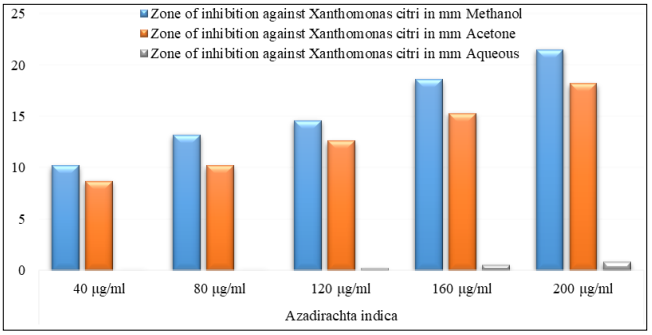

Fig 1.2: *In-vitro* antibacterial activity of Leaf extract at various concentrations against *Xanthomonas citri pv. citri*

References

1. Ahlawat B, MO Jagdish. Medicinal uses of neem (*Azadirachtaindica*): a review article. *World journal of pharmaceutical and medical research*,2019:5(6):167-170.
2. Ghosh S. Antifungal Activity of Neem (*Azadirachta indica*) Extract Against Candida albicans. Journal of Medicinal Plants Research,2010:4(24):2778-2782.
3. Lopez AP, MO Hayek VR Galindo, NW de Torres. Experimental Design to Determine the Factors Affecting the Preparation of Extracts for Antibacterial Use. *Natural Product Communications*,2008:3(3):2008.
4. Sharma N. Antibacterial Efficacy of Neem (Azadirachtaindica) Leaf Extract against Gram-Positive and Gram-Negative Bacteria. Indian Journal of Natural Products and Resources,2012:3(4);378-384.
5. Shobha V. Antifungal Potential of Azadirachtaindica (Neem) Leaf Extracts Against Dermatophytes. International Journal of Current Microbiology and Applied Sciences,2016:5(7):124-130.
6. Shekhawat PS, Prasada R. Antifungal properties of some plant extracts; inhibition of spore germination. *Indian Phytopathology*,1971:24:800-802.
7. Tripathi P. Bioactive Compounds from Neem: A Potential Therapeutic Agent against Fungal Infections. *Pharmaceutical Biology*,2007:45(8):591-597.