

Original Research Article

Antifungal Activity of leaf extract of Neem (*Azadirachta Indica* Linn)

D.K.Shrivastava and Kshma Swarnkar*

Govt. E. Raghavendra Rao Postgraduate Science College Bilaspur (Chhattisgarh) - 495001, India

*Corresponding author

ABSTRACT

Keywords

Anti-fungal agents,
Azadirachta indica

Azadirachta indica (Neem) leaf extract was taken to test its antifungal activity against three fungal species - *Aspergillus flavus*, *Alternaria solani* and *Cladosporium*. Ethanolic and Methanolic extracts in different concentrations (25%, 50%, 75% and 100%) was prepared and tested against test organisms using disc diffusion method. Ketoconazole was used to compare the toxicity of Neem' leaf extract and its antifungal activity.

Introduction

Azadirachta indica, also known as Neem, Nimtree and Indian Lilac is a tree in the mahogany family - Meliaceae. It is one of two species of the genus *Azadirachta*, and is native to India, Pakistan and Bangladesh, growing in tropical and semi-tropical regions.

Neem is very common tree in India. It is a large evergreen dense tree growing some 10 to 15 meter tall with a girth of about 2-3 meter. The leaves of this are divided into numerous leaflets, each resembling a full-grown leaf. The Neem tree has played an important role in Ayurvedic medicines and agriculture since time immemorial. The earliest documentation of Neem mentioned the fruit, seeds, oil, leaves, roots and bark for their medicinal properties.

In the ancient document 'Carak-Samhita' and 'Susruta-Samhita', the books at the foundation of the Indian System of natural treatment – Ayurveda, the various parts of this tree have many uses so its named in Sanskrit-“*sarva roga nivarini*”, meaning ‘the curer of all ailments. Its twigs provide a chewing stick and are widely used in the Indian sub continent. Earlier studies on Neem have showed that it contains active substances with multiple medicinal properties (Md mohashine Bhuiyan *et al.*, 1997).

Azadirachta indica is commonly used for the treatment of Diabetes and show the potential role of anti diabetic activity (Shravan kumar Dholi, *et al.*, 2011). Aqueous extract of Neem leaf has a good therapeutic potential as anti hyperglycemic

agent in IDDM and NIDDM (Sonia Bajaj and Srinivasan, B. P. 1999). Abu syed Md *et al.*, 2008 suggests that anti-inflammatory effect of Neem extract is less than, as it produced by dexamethasone. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premises (Saseed, *et al.*, 2008, Mahmood, *et al.*, 2010). The Neem seed has also been used as traditional medicine to treat infections conditions especially those involving the eye and ear. Administration of alcoholic extract of Neem' flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potentiality of an ideal anti-fertility agent (Gbotolorun, *et al.*, 2008). The aqueous extract of Neem has a powerful chemotherapeutic and viral agent (Hassan, *et al.*, 2010).

The purpose of the present study was to assess the antifungal activity of Neem leaves against pathogenic fungi like - *Aspergillus flavus*, *Alternaria solani* and *Cladosporium*.

Materials and Methods

The plant of Neem (*Azadirachta indica*) was selected for study. Its leaves were collected from college campus. The collected leaves were identified with the help of taxonomic key available in departmental library and confirmed with departmental herbaria.

Leaf extract

The completely dried material was powdered and allowed for successive extraction, with concentration of methanol and ethanol (25%, 50% 75% and 100%). The obtained liquid extracts were stored at 4°C in air tight bottle.

Microorganism

The fungal strains - *Aspergillus flavus*, *Alternaria solani* and *Cladosporium sp.* were used. These strains were isolated from local area.

Disc diffusion method

This method (Kirby Bauer *et al*, 1966) is suitable for organism that grows rapidly over night at 35-37°C. The fungicide (specific concentration) impregnated disc absorbs moisture from the agar and fungicide diffuses in to the agar medium. The rate of extraction of the fungicide from the disc is greater than the rate of diffusion, as the distance from the disc increases. There is a logarithmic reduction in the fungicide concentration. Zone of inhibition of fungus growth around each disc is measured and the susceptibility is determined.

Medium

Sabouraud dextrose agar was prepared, autoclaved at 121° C for 15minutes at 15lbs and poured in sterile petri-plates up to a uniform thickness of approximately 5-6mm and the agar was allowed to set at ambient temperature and used.

Inoculums

The fungus were inoculated in sabouraud dextrose agar and incubated at 37o C and all thre fungal species were used as inoculums. Point of inoculums was inoculated over the sabouraud dextrose agar medium, using sterile inoculums loop. After few minute, four disc loaded with 25%, 50%, 75% and 100% methanolic and ethanolic extract were kept at equal distance in each petri-plate.

Table.1 Inhibition of mycelial growth at different concentration of Leaf extracts

Solvent Used for extraction	Fungal Species	Zone of inhibition (mm)				
		Concentration of the extract				0% (Control)
		25%	50%	75%	100%	Positive Control
Methanol	<i>Aspergillus flavus</i> ,	1	1.4	2.0	4.0	0
	<i>Alternaria solani</i>	0.6	0.8	0.9	1.0	0
	<i>Cladosporium</i>	0.1	0.3	0.5	0.6	0
Ethanol	<i>Aspergillus flavus</i> ,	0.3	0.5	0.8	1.0	0
	<i>Alternaria solani</i>	0.2	0.3	0.5	0.6	0
	<i>Cladosporium</i>	0.1	0.2	0.5	0.6	0

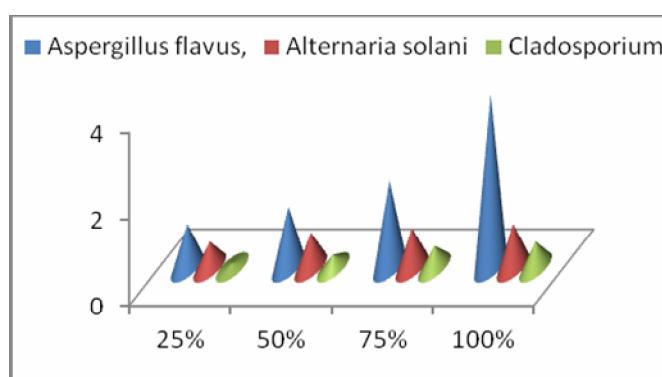


Fig.1 Comparative view if inhibition zone made by various zone made by methanolic extract on different fungal species.

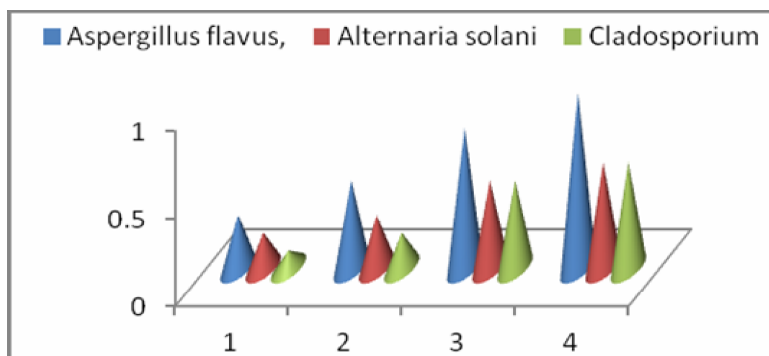


Fig.2 Comparative view if inhibition zone made by various zone made by ethanollic extract on different fungal species

Ketoconazole solution (100% concentration) was added in another plate. Plates were incubated at 37°C for 24hrs. Anti-fungal activity was evaluated by measuring zone of inhibition by using Himedia zone scale.

Results and Discussion

The methanol and ethanollic extract of *Azadirachta Indica* against *Aspergillus flavus*, *Alternaria solani* and *Cladosporium* was found growth

inhibitory, as the zone of inhibition were observed and measured size of ZOI has been incorporated in table, also presented in Fig. 1 & 2 . Among all the extracts the most effective extract - methanolic extract of *Azadirachta Indica* against *Aspergillus flavus* has been observed.

Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects (Srivastava *et al.*, 2000). *Azadirachta indica* leaves possessed good anti fungal activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care (Saradha jyothei, Subbarao 2011). The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the harmful fungus. The phyto-constituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000).

Acknowledgement

Authors are grateful to the Principal, Govt. E. Raghavendra Rao Postgraduate Science College, Bilaspur (C.G.) for providing the required laboratory facility and also thankful to HOD, Dept. of Botany and staff of the department of Microbiology for their supports during present investigation.

References

- Abu. Syed Md. Mosaddek and Md. Mamun Ur Rashid 2008 A comparative study of Anti-inflammatory effect of aqueous extract of Neem leaf and dexamethasone. *Bangladesh J Pharmacol* 3: 44-47.
- A.M. EI- Mahmood, O.B Ogbonna and M.Raji 2010 The antibacterial activity of *Azadirachta indica* Neem associated with eye and ear infections. *Journal of medicinal plant Research*, 414.1414-1421.
- Gbotolorun S.C. Osinubi A.A., Noronha C.C., Okanlawon A.O 2008 Antifertility potential of Neem flower extract on adult female Sprague Dawley rats. *African Health Science* 83: 168-173.
- Hassan Amer, Wafaa A. Helmy, Hanan A.A Taie 2010 *in vitro* Antitumour activities of seeds and leaves Neem *Azadirachta indica* extracts. *International journal of Academic research*. 22, 165-171. Md Mohashine Bhuiyan, Michiko Nishimura seishi.
- Hafiza, R.E. 2000 Peptides antibiotics, *Lancet* 349: 418- 422.
- Md Mohashine Bhuiyan, Michiko Nishimura seishi matsumura and Tsutomu shimono 1997 Antibacterial effects of the crude *Azadirachta indica* Neem bark extract on *Streptococcus sobrinus*, *Pediatric dental journal* 71:61-64.
- Saradhajyothei Koona, Subbarao Budida 2011 Antimicrobial potential of the extracts of the leave of *Azadirachta indica* , *Linn. Nat Sci Biol*, 31 65-69.
- Saseed A. Khan and Junaid Aslam 2008 Study on the effect of Neem *Azhadirachta indica* leaves smoke incontrolling airborne Bacteria in Residential premises. *Current research in Bacteriology* 1 2: 64-66.
- Shravan Kumar Mankala, Kannappan Nagappan 2011 *in vivo* Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. *Journal of applied Pharmaceutical science*, 7, 100-105.
- Sonia Bajaj, Srinivasan B.P. 1999 Investigation into the Anti diabetic activity of *Azadirachta indica*. *Indian journal of pharmacology* 31:138-141.
- Srivastava A Shukla Kumar YN 2000 Recent development in plant derived antimicrobial constituents A Review. *J Med Arom Pl. Sci*.20: 717-72.