

## Antifungal Activity of leaf extracts of *Nerium indicum* against Pathogenic Fungi *Aspergillus spp.*

Renu Jangid<sup>1</sup>, Tahira begum<sup>2</sup>

<sup>1,2</sup>Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India

Available online at: [www.isroset.org](http://www.isroset.org)

Received: 11/May/2019, Accepted: 20/Jun/2019, Online: 30/Jun/2019

**Abstract-** Medicinal plants represent a rich source of antimycotic agents. Plant extract has been used traditionally to treat a number of infectious diseases. Medicinal plant *Nerium indicum* belongs to the family Apocynaceae. The leaf extracts of *Nerium indicum* exhibited significant antifungal activity against pathogenic fungi (*A. niger*, *A. flavus* and *A. fumigatus*). *Aspergillus species* were isolated from soil samples. The soil samples collected from Ajmer, Rajasthan (India). Antifungal activities of the different leaf extracts were compared with the zones of inhibition produced by the standard antibiotic (Gresiofulvin). The ethanol, aqueous and DMSO extracts of *Nerium indicum* showed high antifungal activity against *Aspergillus species* except ethanol extract show lowest activity against *A. niger*. These results support the leaf extract of *Nerium indicum* can be used to cure fungal infections in both plant and human beings.

**Keywords:** Plant extracts, Disc diffusion, Antifungal activity, *Aspergillus species*.

### I. INTRODUCTION

Medicinal plants are represent a rich source of antifungal agent and are traditionally used for the treatment of infectious diseases. Plants and their products have been used since ancient times for medicinal purpose. According to a report by the WHO (World Health Organization) 20,000 plant species are currently in use for medicinal purposes [1]. Plant extracts has been used traditionally to treat a number of infectious disease including those caused by bacteria, fungi, protozoa and viruses. Microorganisms have developed resistance to many antibiotics and as a result, an immense clinical problem in the treatment of infectious diseases has been created [2]. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more frequent [3, 4]. Research on new antimicrobial substances should be continued and small molecules from medicinal chemistry, as well as natural products are still major sources of innovative therapeutic agents for infectious disease [5]. Natural products are generally harmless or have minimum side effects as compared to synthesis drugs [6]. Aqueous and solvent extracts of different plant parts have been tested for pharmacological and therapeutic activities, such as antimicrobial, hepatoprotective and other activities [7]. Testing plant extracts for antifungal activity could be a good source to identify new antifungal drugs. The search for novel antifungal agents relies in great parts on ethno medicinal plants information and ethno pharmacologic exploration [8]. A vast array of diseases occurs due to the fungal infections such as athlete's foot, candidiasis, aspergillosis and

zygomycosis, etc. Aspergillosis is an infection caused due to inhalation of *Aspergillus* spores. *Aspergillus species* are ubiquitous in organic matter such as hay, decaying vegetation, soil and construction sites. The types of health problems caused by *Aspergillus* include allergic reactions, lung infections, and infections in other organs. In plants also *Aspergillus species* cause harmful disease such as black rot, collar rot disease etc.

*Nerium indicum* is an important medicinal plant of family Apocynaceae and commonly known as "Kaner" in hindi and "Karabi" in Bengali. It grows upto 2-6m in height. Leaves are long, thick, leathery, simple, whorled, linear lanceolate, hairless, 9-14 cm in length with horizontal nerves. In medicinal plants *Nerium indicum* is one of the plants which are famed for its therapeutic efficiency in different disease globally. It is used as traditionally medicine in different parts of the world, especially in India and China. The Ethanol flower extracts of this plant were showed antifungal activity against different fungal pathogens [9]. In the present study, leaf extracts of *Nerium indicum* has been chosen for the investigation of in vitro antifungal activity against *Aspergillus niger*, *Aspergillus fumigates*, *A. flavus*.

The aim of this study was to assess the antifungal activity of leaf extracts of *Nerium indicum* against *Aspergillus sp.* From the above discussion were that the plant products have powerful antifungal properties, which can be used for the treatment and were the various ailments.

## II. MATERIAL AND METHODS

### 2.1 Fungal Material

Three *Aspergillus fungi* selected for antifungal activity. All three cultures were isolated from soil samples by Hair Baiting technique.

### 2.2 Culture Media

The isolated fungus was maintained on Sabouraud's Dextrose Agar medium.

### 2.3 Plant Material

The leaves of *Nerium indicum* were collected during spring and summer seasons (April-June, 2017.) in the local area of Ajmer district, Rajasthan and authenticated by Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India. The leaves were washed thoroughly 2-3 times with running water, blotted with filter paper and dried in the shade. After then dried material was ground into powder using blender and sealed in polythene bags for further use.

### 2.4 Preparation of leaf extracts

Plant extract was prepared by soxhlet extraction method. About 10gm of dried and powdered leaf material was uniformly packed in to a thimble and run in soxhlet extractor with ethanol/ DMSO for 48 hours. The extract was then filtered with the help of filter paper and solvent was evaporated from extract. For aqueous extraction, 10gm of powdered material was macerated by blender with 100ml of distilled water and solvent powder mixture was kept at room temperature for 48 hours, the extract was filtered through filter paper. The extracts were kept in refrigerator at 4°C for further experiments.

### 2.5 Antifungal Activity

Antifungal activities of the leaf extracts were determined, using the Disk diffusion method as described Kirby-Bauer (1956). Filter paper Discs of 6mm diameter were soaked

with 1ml of extracts. Sabouraud's Dextrose Agar plates were inoculated with each fungal culture by point inoculation. The plates were done in triplicates and were incubated at 27°C. The antifungal activity was taken on the basis of diameter of zone of inhibition, which was measured after 7 days of incubation and the mean of three readings is presented.

### 2.6 Control experiment

The presence of inhibition of the treated fungus was calculated using Griseofulvin as standard.

### 2.7 Statistical analysis

The data were calculated as mean±SD and analyzed using one way analysis of variance (ANOVA).

## III. RESULTS

The data on *Aspergillus species* isolated from soils of Ajmer district (Rajasthan). Antifungal activity of different extracts against pathogenic fungi (*Aspergillus niger*, *A. flavus*, and *A. fumigates*) was evaluated by the Disc diffusion method. The results and screening of antifungal activity of *Nerium indicum* leaf extracts are summarized in table 1. All the extracts tested exhibited different degrees of antifungal activity against *A. niger*, *A. flavus* and *A. fumigates*. *Nerium indicum* shown high antimycotic activity against all the tested fungus except *A. niger*. The percentage of growth inhibition of the selected pathogenic *Aspergillus species* by leaf extracts of *Nerium indicum* was recorded and the data were showed in table 2. The percentage growth inhibition of pathogenic fungi by leaf extracts were data put in graph present in Fig.1. The graph showed that aqueous extract of *Nerium indicum* leaf exhibited strong antifungal against *A. niger* with inhibition zone of 16mm diameter as compared with inhibition zone of 14mm diameter produced by commercially available standard antibiotic (Griseofulvin). The lowest activity was recorded DMSO extract against *A. flavus* with inhibition zone of 16mm diameter.

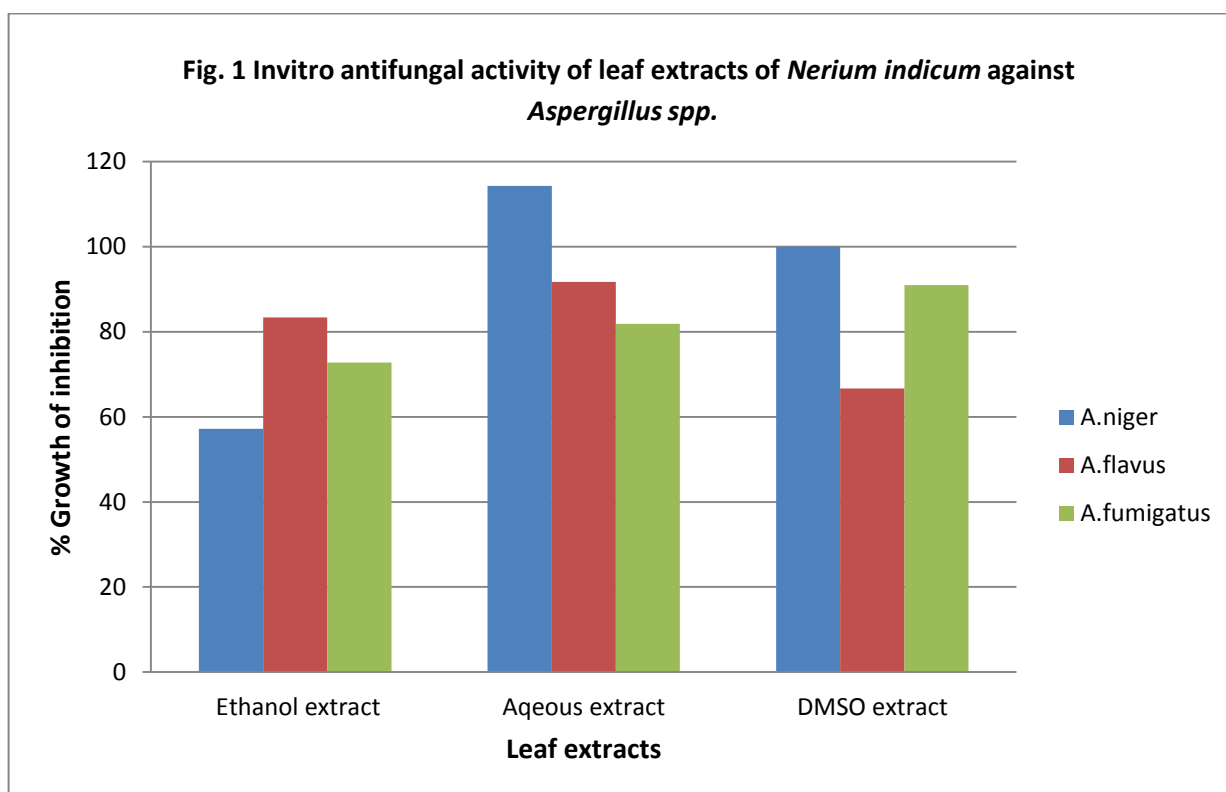
**Table 1: Antifungal activity of different solvent extracts of *Nerium indicum* leaves.**

Extracts/drug	Zone of inhibition (mm)		
	<i>A. niger</i>	<i>A. flavus</i>	<i>A. fumigates</i>
Ethanol	08±0.58	20±0.58	16±1.16
Aqueous	16±1.16	22±1.16	18±0.58
DMSO	14±1.16	16±0.58	20±0.58
Griseofulvin	14±0.58	24±1.16	22±0.58

Activity index was calculated as the mean value of net zones of inhibition (mm) against three *Aspergillus species*.

**Table 2:** The % inhibition of the of different plant extracts compared to Griseofulvin (100% inhibition) against pathogenic *Aspergillus species*.

Pathogenic fungus	Griseofulvin	Ethanol	Aqueous	DMSO
<i>A. niger</i>	100	57.15	114.28	100
<i>A. flavus</i>	100	83.34	91.67	66.67
<i>A. fumigatus</i>	100	72.73	81.82	90.91



#### IV. DISCUSSION

Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health. It's important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [12]. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine a natural blueprint for the development of new drugs [13]. In our study we found that leaf extracts of *Nerium indicum* demonstrated strong antifungal activity on *Aspergillus species*. Antifungal activity of leaf extracts (ethanol, aqueous and DMSO) were representing in table 1. Better antifungal activity was observed against *A.niger* with aqueous extract (zone diameter of 16mm) as compared standard (zone diameter of 14mm). The antifungal activities of the leaf extract obtained using different solvent were compared with that of Griseofulvin and the % of inhibition was calculated. (Table 2).

#### V. CONCLUSION

The eventual conclusion of this study supports the traditional medicine use of leaf extracts in treating different infections caused by pathogenic *Aspergillus fungi*. Currently microbial infectious have become an important clinical threat, with significant associated morbidity and mortality which is mainly due to the development of microbial resistance to existing of

microbial agents [14]. As a positive control in our study, the leaf extracts of *Nerium indicum* demonstrated influential activity against *Aspergillus species*.

### ACKNOWLEDGMENT

The authors are grateful to Dr. Rama Rani Jain for confirming the identify of plant and *Aspergillus sp.* We are also thankful to Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan. For providing facilities and CSIR-JRF for providing financial support.

### REFERENCES

- [1]. Scorzoni L., Benaducci T., Fusco-Almeida A. M., Siqueira Silva D. H., Silva-Bolzani V., Mendes-Gianinni M. J. S. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida sp.* And *Cryptococcus sp.* Brazilian Journal of Microbiology, 38:391-397, 2007.
- [2]. Davies, J. Inactivation of antibiotic and the dissemination of resistance genes. *Sci.*, 264: 375-382, 1994.
- [3]. Fleming, R.V., Walsh, T.J., Anaissie, E.J. Emerging and less common fungal pathogens. *Infectious Disease Clinics of North America* 16, 915-933, 2002.
- [4]. Walsh, T.J., Groll, A. H. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transplant Infectious Disease* 1, 247-261, 1992.
- [5]. Clardy, J. ; Walsh, C. Lessons from natural molecules. *Nature*, 432(7019): 829-37, 2004.
- [6]. Bhadauria S., and Kumar P. In vitro antimycotic Activity of Some Medicinal Plants Against Human Pathogenic Dermatophytes. *Indian journal of fundamental and Applied Life Sciences* 2231-6345, 2011.
- [7]. Anjana S, Rani V, Padmini R : Antibacterial activity of some medicinal plants used by Tribals against UTI causing pathogens. *Wo Appl Sci J* 2009; 7; 332-339.
- [8]. Webster D., Taschereau P., Belland R.J., Sand c., Rennie R.P. Antifungal activity of medicinal plant extracts; preliminary screening studies, 2007.
- [9]. Hadizadeh I, Peivastegan B, Kolahi M. Antifungal activity of nettle (*Urtica dioica L.*), colocynth (*Citrullus colocynthis L. Schrad*), oleander (*Nerium oleander L.*) and Konar (*Zizyphus spina-christi L*) extracts on plants pathogenic fungi. *Pak J Biol Sci.* 2009; 12:58-63. [PubMed] [Google scholar].
- [10]. Balandrin M F, Klocke JA, Wurtele E S and Bollinger W H: Natural plant chemicals: Sources of industrial and Medicinal materials. *Science* 1985; 228:1154-1160.
- [11]. Alzoreky N S, Nakahara K: Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int J Food Microbial* 2003; 80; 223-230.
- [12]. Mitscher, L.A., S. Drake, S.R. Gollapudi and S.K. Okwute. A modern look at folkloric use of anti-infective agents. *J. Natural Products*, 50: 1025-1040, 1987.
- [13]. Jawed, S. and M. Ali. Antimicrobial activity in higher plants, *Hamdard medicuse*, 45:71-74, 2002.
- [14]. Balouiri M., Sadiki M., Ibnsouda S. K. Methods for invitro evauading antimicrobial activity: A review. *Journal of Pharmaceutical Analysis.* 71-79, 2016.

### Author's profile

Ms. Renu Jangid is a research scholar, Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India. She has completed M.sc. Degree in Botany from Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India.

Dr. Tahira Begum is currently working as Associate Proffessor, Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India. She has awarded with a Ph.D degree in Botany from Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India.