Antifungal Activity of Leaf Extracts of Selected Medicinal Plants Against Aspergillus Species.

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***Abstract*—Plant products are a rich source of antifungal agents and are traditionally used to treat many fungal infectious diseases. In vitro, the antifungal activity of leaf extract of *Catharanthus roseus, Lantana camara, Sida cordifolia* and *Ziziphus mauritiana* were studied against *Aspergillus sp. (A. flavus* and *A. fumigatus,* and *A. niger). Aspergillus* species isolated from soil samples were collected from Ajmer City, Rajasthan (India). Antifungal activities of the different leaf extracts were compared with the zones of inhibition produced by the standard antibiotic (Gresiofulvin) treated as a control. In the present research, three solvents including aqueous, ethanol, and DMSO (dimethyl sulfoxide) were prepared as leaf extracts for antifungal activity. All leaf extracts of selected medicinal plants recorded significant activity against *Aspergillus* species. However, the aqueous and ethanol leaf extracts of all the selected medicinal plants showed the highest antifungal activity against all the *Aspergillus* species.**

***Index Terms*—Plant extracts, Antifungal activity, Aspergillosis, leaf extracts, Disc diffusion method.**

I. INTRODUCTION

Plant and their product have been used since ancient times for medicinal purposes. According to the WHO (World Health Organization), as many as 80% of the World population depends on traditional medicine for their primary healthcare need. According to a report, 20,000 plant species are currently using it for medical purposes. A plant is said to be medicinal when at least one-part possess therapeutic properties [1].

In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure infectious diseases. A large number of medicinal plants are used in various medicinal practices in India [2, 3]. Fungal infections pose a serious public health risk at a global level. There are an estimated 1.5 million fungal species, of which over 8,000 are known to cause plant diseases and around 300 are pathogenic to humans. Fungal pathogens impact several hosts and can cause both human and plant diseases. *Aspergillus* is an opportunistic fungal pathogen belonging to phylum Ascomycota, that is known to cause severe human diseases including aspergillosis and otomycosis [4]. Aspergillosis caused by inhaling spores from the *Aspergillus* genus which is commonly found in the environment. While most people can breathe in these spores without adverse effects, those with weakened immune systems or pre-existing lung conditions are at a significantly higher risk of developing serious health issues related to this infection. Awareness of its types, symptoms, and treatment options is crucial for effective management and improved this fungal infection [5].

The genus *Aspergillus* was identified by Micheli in 1729. There are about 200 species of *Aspergillus* in nature, but only around 20 are known to cause human disease. *Aspergillus flavus, A. fumigatus, and Aspergillus niger* are the three most frequent pathogenic agents, accounting for approximately 95 percent of all cases. *Aspergillus* are generally saprobic and found in soil; however, some have been shown to have a positive or negative impact on human activities [6]. In humans, *Aspergillus* infections predominantly affect the respiratory system. Inhaled *Aspergillus* spores can germinate under optimum lung circumstances such as high humidity, oxygen, and carbon dioxide. *Aspergillus fumigatus* is the most common aspergillosis-causing species, followed by *A. flavus* and *A. niger. Additionally, A. niger* is also known to be one of the most common infection-causing fungal pathogens that cause mycosis in humans. The relative data suggests that these Molds can cause systemic invasive infections, killing at least 50% of those affected [7].

These broad-spectrum antimycotic agents are much costlier and possess harmful side effects. Therefore,

the human population in the present era is more attracted to plant-derived medicine. Natural products are generally harmless or have minimum side effects as compared to synthetic drugs. Fungal infections are the leading cause of death in both advanced and developing countries. This is due to the overuse of immunosuppressive treatments, long-term antibiotics, and longer survival of immunocompromised individuals [8].

II. MATERIALS AND METHODS

A. Fungal isolation

Aspergillus flavus, A. fumigatus, and *A. niger* were isolated from soil samples by the Hair Baiting technique of Vanbreuseghem (1952) and processed for antifungal activity [16]. The fungal cultures of *Aspergillus* were maintained on Sabouraud Dextrose Agar medium with the antibiotic Streptomycin [50µg/L]. Plates were incubated for 7 days at 27°C. The colonies of *Aspergillus* were transferred to new SDA media plates for purity and to avoid contamination.

B. Plant material

The leaves of selected four medicinal plants including *Catharanthus roseus, Lantana camara, Sida cordifolia,* and *Ziziphus mauritiana* were collected from Ajmer district, Rajasthan, India. The plants were identified and authenticated by the plant taxonomy expertise of the Department of Botany, Samrat Prithviraj Chauhan Government College Ajmer, Rajasthan, India. The leaves were washed thoroughly 2-3 times with normal tap water followed by distilled water for the removal of debris and unwanted microbial communities. The leaves were subjected to drying followed by fine grinding for further procedure.

C. Preparation of leaf extracts

The leaf extracts were prepared using the Soxhlet extraction method. About 20g of dried and powdered leaf material was packed uniformly into a thimble and processed in a Soxhlet extractor with three individual solvents (1:10 w/v) mainly aqueous, ethanol, and DMSO for 48 hours. The processed extract was filtered using Whatman filter paper no. 1, and the solvent was evaporated. The percentage crude yield extracts were determined. The extracts were kept in the refrigerator at 4℃ and used to determine antifungal activity against *Aspergillus* species [17]. The preliminary phytochemical testing of leaf extracts to detect the presence of different secondary metabolites using standard potocols.

D. Antifungal activity

The antifungal activity of the leaf extracts was evaluated using the disk diffusion method described by Kirby-Bauer [18]. Autoclaved filter paper discs of 6 mm diameter were soaked into individual solvent extracts measuring 1 ml in sterile petri plates (100 mm). Each fungal inoculum was inoculated by the point inoculation technique. The experiment plates were prepared in triplicate and incubated at 27℃. The antifungal activity was assessed using the diameter of the zone of inhibition, which was measured after 7 days of incubation, and the average of three measurements was reported. For controls, commercial antibiotic discs of Griseofulvin were used following the same protocol as treatments.

E. Statistical analysis

For antifungal bioassay, data analysis was done by measuring the colony diameter of fungal growth. Descriptive analysis and one-way analysis of variance (ANOVA) were conducted to study the significant differences among treatments and corresponding controls. Tukey's HSD test was conducted to evaluate the significant differences among the different groups. IBM SPSS software was used for the statistical analysis.

III RESULTS AND DISCUSSION

A. Isolation and Identification of *Aspergillus species*

Aspergillus species were isolated from Ajmer district, Rajasthan, India. The soil samples were collected by the Hair baiting technique. Fungal morphology was studied by the growth pattern and pigmentation of fungal colonies. Microscopic examination of fungal colonies was done by characteristic macroconidia and microconidia through staining with Lactophenol cotton blue (Fig. 1).

B. Preliminary Detection of Phytocompounds

The preliminary detection of phytochemical present in leaf extracts of *C. roseus, L. camara, N. indicum, Z. mauritiana,* and *S. cordifolia* showed positive

C. Antifungal activity of leaf extracts

The antifungal activity of leaf extracts was determined by measuring the diameter of the zone of inhibition observed using the Disc diffusion method. Leaf extracts of selected plants showed potential antifungal impact against the isolated pathogenic fungi including *A. flavus, A. fumigatus, and A. niger*. The leaf extracts prepared in all three solvents showed a positive to neutral impact against the growth inhibition of selected fungal species. However, the efficacy of aqueous extracts showed an overall positive effect on the growth retardation of *Aspergillus* species. Aqueous extract followed by ethanol showed a positive effect on the growth retardation (Table-1; Fig. 2). The highest impact of ethanol extract was observed from *Z. mauritiana* with a colony diameter of 21±1 followed by *L. camara, S. cordifolia*, and *C. roseus* with 19±1, 19±1, and 18±1. Kumari and Gupta, (2013) studied the antifungal activity of *Catharanthus roseus* against various clinically significant fungal strains including *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Fusarium moniliforme*. three extraction media mainly ethanol, acetone, and aqueous were used for this study. However, the maximum inhibition was observed in *F. moniliforme* [19]

For *Aspergillus* species, *A. niger* showed a positive response to the plant extracts followed by *A. fumigatus*, and *A. flavus*. Contrary, the control Griseofulvin disc, showed a neutral impact on the three species selected. The results showed an overall positive impact with a significant difference (P<0.05) against all the three solvents tested. However, DMSO extracts of *Z. mauritiana* showed no significant difference in comparison to the control tested with an average colony diameter of 12±1. Girish, K. (2017) evaluated the antimicrobial activity of crude methanolic and acetone extracts of *Lantana camara* against thirteen bacteria and eight fungus species. Both solvent extracts exhibited maximum growth inhibition against *Staphylococcus aureus* and *Alternaria alternata* [20].

Similarly, Zenat, M. et. al., (2024) investigated the antifungal activity of methanolic leaf extracts of *Azadirachta indica, Lantana camara, Wedelia chinensis, Moringa oleifera,* and *Coccinia grandis.* The most effective treatments in suppressing the growth of *Aspergillus* and *Penicillium* were shown by *Azadirachta indica, Moringa oleifera*, and *Lantana camara* extracts [21]. Methanol leaf extracts of *Sida cordifolia, Ziziphus mauritiana, Acacia nilotica, Tinospora cordifolia,* and *Withania somnifer* demonstrated significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus,* and *Xanthomonas axonopodis malvacearum*, as well as antifungal activity against *Aspergillus flavus, Dreschlera turcica*, and *Fusarium verticillioide.* [22]

Table 1. Antifungal activity of leaf extracts prepared in three solvents of four medicinal plants against *Aspergillus sp*.

Average measurements of colony diameter (cms) were studied for evaluation. The values represent mean±SE.

Fungal species		Zone of Inhibition (cm)				
	Solvent	Control	*C. roseus*	*L. camara*	*S. cordifolia*	*Z. mauritiana*
A. Niger	Aqueous	7±0	16±1	19±0	11±1	22±0
	Ethanol	10±0	19±1	12±1	20±1	10±0
	DMSO	6±0	9±0	9±1	8±1	9±1
A. fumigatus	Aqueous	8±1	20±0	20±1	20±1	23±1
	Ethanol	8±1	12±1	15±0	11±1	21±1
	DMSO	15±1	17±1	17±1	15±0	15±1
A. Flavus	Aqueous	15±0	17±1	17±0	17±0	17±1
	Ethanol	8±0	13±1	17±1	16±0	17±1
	DMSO	10±0	15±1	15±1	16±0	14±1

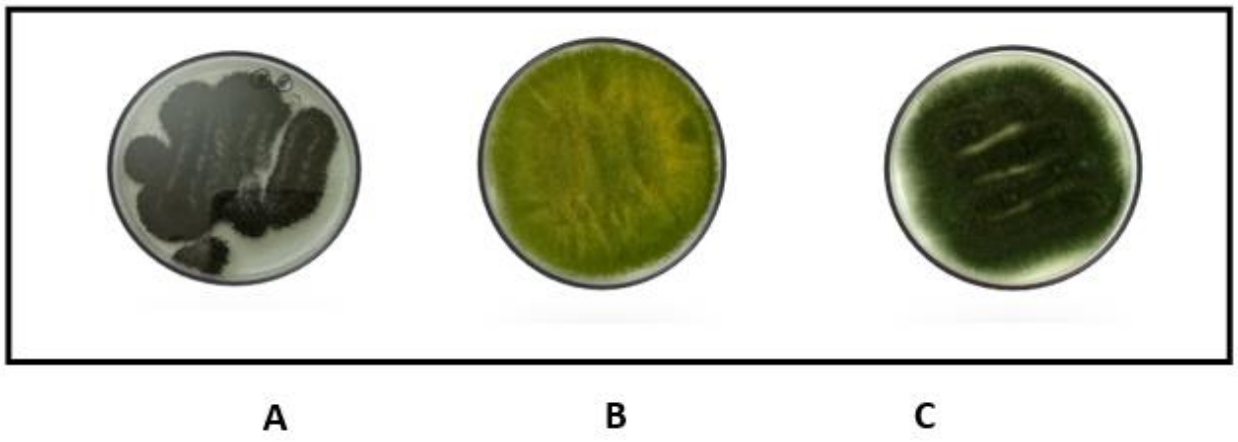

Figure 1. Pure culture of fungal growth of Aspergillus.
A. *Aspergillus niger*, B. *Aspergillus flavus*, C. *Aspergillus fumigatus*.

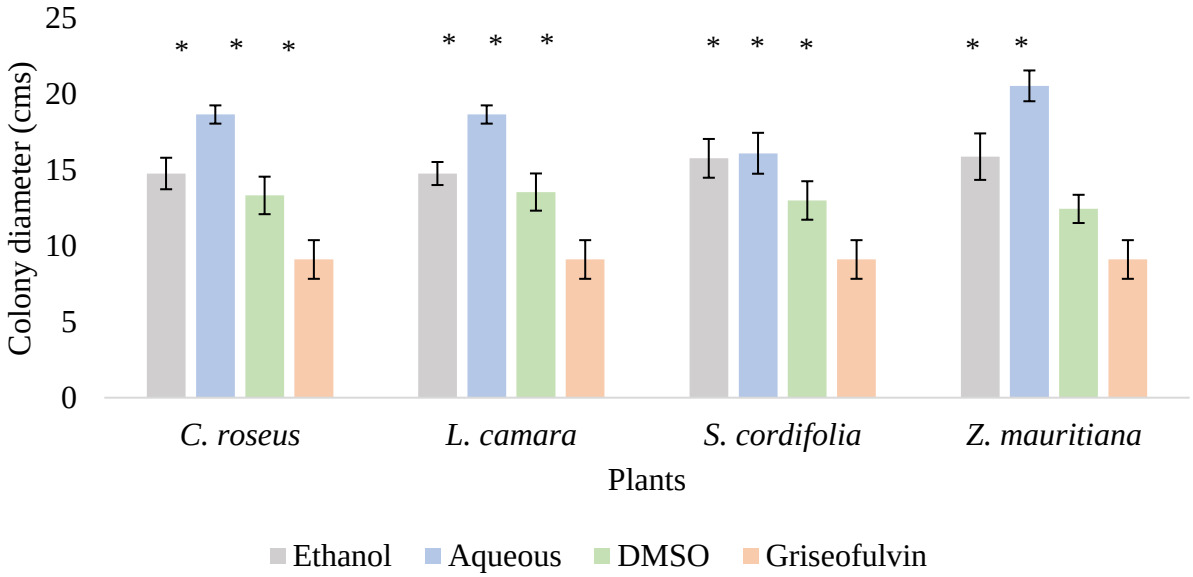

Figure 2. Effect of leaf extracts on growth inhibition of *Aspergillus* species.
Three solvents were used for preparing plant extracts. The results for each solvent show the average against each fungal species tested. The commercial antifungal disc of Griseofulvin was taken as control. * Indicates $P<0.05$ based on ANOVA and Tukey's HSD test. The values are Mean ± SE of triplicates ($P<0.05$).

Plants have provided ample source of novel drugs as plant derived medicines have made significant contribution towards human health. All the leaf extracts tested exhibited different degrees of antifungal activity. The result of the present investigation indicate that antifungal activity varies with the species of plants. Aqueous and ethanol extracts showed effective inhibitory activity in comparison to DMSO against selected *Aspergillus sp.* Thus, the study establishes the value of plant products used in Ayurveda, which may be of significant interest in the development of novel medications.

VI. CONCLUSIONS

Plants are a valuable source of potentially useful products for the development of new chemotherapeutic agents. Plants produce a variety of medicinal drugs that can inhibit the growth of microorganisms and are a rich source of innovative pharmaceutical products. In past years, chemical

drugs used in the treatment of fungal infections have been known to possess many side effects, and resistance development is common against these drugs. Plant products have been considered a traditional source of antimicrobial medicines for the past few decades. This research shows the investigation of different solvents (aqueous, ethanol, and DMSO) as antifungal agents. All three solvents have been tested against *Aspergillus sp*. isolated from soil samples. All leaf extracts of selected medicinal plants demonstrated influential positive responses against *Aspergillus* species. The natural products have not any side effects and also, they are cheaper. Thus, plant products specifically medicinal plants could be one of the best sources of natural drug alternatives for human and animal health. Therefore, this study focuses on the antifungal properties of leaf extracts against pathogenic *Aspergillus* species. These properties are used to develop natural drugs.

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VI. DISCLOSURE STATEMENT

We declare that we have no conflict of interest.

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VIII. AUTHOR CONTRIBUTIONS STATEMENT

Renu Jangid structured and wrote the manuscript. Additionally, the laboratory experiments, data structure, and concept design were also conducted by Renu Jangid. Shruti Ojha is responsible for statistical analysis, critical revision, and data interpretation of the manuscript.

REFERENCES

[1] A. K. Dhiman, Sacred plants and their medicinal uses. Daya Publishing House New Delhi, India, 2003.

[2] B. Patwardhan, A. D. Vaidya, and M. Chorghade, "Ayurveda and natural products drug discovery," Current science, pp. 789-799, 2004.

[3] P. K. Mukherjee and A. Wahile, "Integrated approaches towards drug development from Ayurveda and other Indian system of medicines," Journal of ethnopharmacology, vol. 103, no. 1, pp. 25-35, 2006.

[4] E. Schuster, N. Dunn-Coleman, J. Frisvad, and P. Van Dijck, "On the safety of Aspergillus niger–a review," Applied microbiology and biotechnology, vol. 59, pp. 426-435, 2002.

[5] G. d. Hoog, J. Guarro, J. Gene, and M. J. Figueras, Atlas of clinical fungi (no. Ed. 2). 2000.

[6] J. Houbraken, R. P. de Vries, and R. A. Samson, "Modern taxonomy of biotechnologically important Aspergillus and Penicillium species," Advances in applied microbiology, vol. 86, pp. 199-249, 2014.

[7] A. H. Hashem et al., "Antifungal activity of biosynthesized silver nanoparticles (AgNPs) against aspergilli causing aspergillosis: Ultrastructure Study," Journal of Functional Biomaterials, vol. 13, no. 4, p. 242, 2022.

[8] G. Molero et al., "Candida albicans: genetics, dimorphism and pathogenicity," International Microbiology, vol. 1, no. 1, pp. 95-106, 1998.

[9] C. A. Jaleel, R. G. Ragupathi Gopi, and R. P. Rajaram Panneerselvam, "Alterations in non-enzymatic antioxidant components of Catharanthus roseus exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens," 2009.

[10] L. Muhammad, N. Muhammad, A. Tanveer, and S. Baqir, "Antimicrobial activity of different extracts of Catharanthas roseus," Clin. Exp. Med. J, vol. 3, pp. 81-85, 2009.

[11] B. Sharma and P. Kumar, "Bioefficacy of Lantana camara L. against some human pathogens," Indian Journal of Pharmaceutical Sciences, vol. 71, no. 5, p. 589, 2009.

[12] H. Iqbal et al., "Extracts of Sida cordifolia contain polysaccharides possessing

immunomodulatory activity and rosmarinic acid compounds with antibacterial activity," BMC complementary medicine and therapies, vol. 22, no. 1, p. 27, 2022.

[13] M. Sivadasan and N. A. Kumar, "Sida ravii, a new species of Malvaceae from India," Willdenowia, pp. 651-654, 1996.

[14] H. B. Said Gulam Khan, N. I. Mohd Sarmin, M. H. Arzmi, H. F. Amiruddin, and A. A. Mohd Radzi, "Antifungal activities of Ziziphus mauritiana against Candida albicans: in vitro study," Compendium of Oral Science (COS), vol. 7, pp. 1-12, 2020.

[15] D. Dahiru, J. Sini, and L. John-Africa, "Antidiarrhoeal activity of Ziziphus mauritiana root extract in rodents," African journal of biotechnology, vol. 5, no. 10, 2006.

[16] R. Vanbreuseghem, "Technique biologique pour l'isolement des dermatophytes du sol," Ann Soc Belge Med Trop, vol. 32, no. 2, pp. 173-178, 1952.

[17] A. R. Abubakar and M. Haque, "Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes," Journal of Pharmacy and Bioallied Sciences, vol. 12, no. 1, pp. 1-10, 2020.

[18] A. W. Bauer, D. M. PERRY, and W. M. KIRBY, "Single-disk antibiotic-sensitivity testing of staphylococci: An analysis of technique and results," AMA archives of internal medicine, vol. 104, no. 2, pp. 208-216, 1959.

[19] K. Kumari and S. Gupta, "Antifungal properties of leaf extract of Catharanthus roseus l (g.) Don," American Journal of Phytomedicine and Clinical Therapeutics, vol. 1, no. 9, pp. 698-705, 2013.

[20] K. Girish, "Antimicrobial activities of Lantana camara Linn," Asian Journal of Pharmaceutical and Clinical Research, vol. 10, no. 3, pp. 57-67, 2017.

[21] M. Zenat et al., "Antifungal activity of various plant extracts against aspergillus and penicillium species isolated from Leather-Borne Fungus," Microbiology Research Journal International, vol. 34, no. 1, pp. 10-23, 2024.

[22] R. G. Tantiado, "Survey on ethnopharmacology of medicinal plants in Iloilo, Philippines," International Journal of Bio-science and Bio-technology, vol. 4, no. 4, pp. 11-26, 2012.