Comparative Antifungal efficacy of Ashoka tree extract using polar and non-polar solvents against Aspergillus niger, Trichoderma viridens and Candida albicans

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Abstract

This study investigates the antifungal activity of polar and non-polar solvents against three significant fungal organisms: *Aspergillus niger*, *Trichoderma viridens* and *Candida albicans*. Utilizing various solvents, we assessed their efficacy in inhibiting fungal growth through a series of in vitro assays. The results demonstrated distinct variations in antifungal potency, with polar solvents showing higher inhibition rates against *A. niger* and *C. albicans*, while non-polar solvents exhibited greater efficacy against *T. viridens*. Our findings highlight the importance of solvent polarity in antifungal treatments and suggest potential applications in developing targeted antifungal therapies. Further research into the mechanisms of action is warranted to optimize these treatments for clinical use.

Key words:
Polar solvents, Non-polar solvents, Solvent efficacy, Fungal inhibition, Antifungal therapies

Introduction

Fungal infections pose a significant threat to human health, agriculture, and food security. Among the plethora of fungal pathogens, Aspergillus niger, Trichoderma viridens, and Candida albicans stand out due to their ubiquitous presence and their role in various diseases and spoilage (Bennett & Klich, 2003). Effective management of these pathogens is essential, necessitating ongoing research into novel antifungal agents and treatment strategies.

The choice of solvent in antifungal studies is critical, as it can significantly influence the efficacy of the antifungal agents (Ali et al., 2011). Solvents are broadly classified into polar and non-polar, each with distinct properties that affect their interaction with fungal cells and the dissolution of antifungal compounds (Sangwan et al., 2018). Polar solvents, such as water and methanol, are characterized by their ability to dissolve ionic and other polar substances, enhancing the bioavailability of many antifungal agents (Rizvi & Saleh, 2018). In contrast, non-polar solvents, such as hexane and chloroform, dissolve non-polar substances and can alter the cell membrane integrity of fungi (Saini et al., 2015).

Aspergillus niger is known for its resilience and ability to thrive in diverse environments, often leading to food spoilage and allergic reactions (Schuster et al., 2002). Trichoderma viridens, while beneficial as a biocontrol agent, can also act as an opportunistic pathogen (Harman et al., 2004). Candida albicans, a common cause of fungal infections in humans, poses significant challenges in clinical settings due to its ability to form biofilms and develop resistance to antifungal drugs (Pfaller & Diekema, 2007).

Previous studies have explored the antifungal properties of various solvents and their effects on different fungi. For instance, the antifungal activity of polar solvents has been well-documented, showing significant inhibitory effects against various fungal species (Nazzaro et al., 2013; Suresh et al., 2010). Conversely, non-polar solvents have been studied for their ability to disrupt fungal cell membranes, providing a different mechanism of antifungal action (Prasad et al., 2012; Bhargava et al., 2014).

This study aims to provide a comparative analysis of the antifungal efficacy of polar and non-polar solvents against *A. niger, T. viridens,* and *C. albicans*. By conducting a series of in vitro assays, we seek to elucidate the differential impacts of solvent polarity on fungal inhibition and to identify potential applications in developing more effective antifungal treatments. The findings from this research will contribute to a deeper understanding of the role of solvent polarity in antifungal activity and may inform the selection of solvents in future antifungal formulations.

Material and Methods

The fungal cultures were acquired from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology in Chandigarh, India, as well as from the National Collection of Industrial Microorganisms (NCIM) in Pune, India. The antifungal activity of Ashoka Tree Extract was investigated using fungal cultures of Aspergillus niger (ITCC-6117), Trichoderma viridens (MTCC-167), and Candida albicans (ATCC-20).

Fungal media (potato dextrose agar and sabouraud’s dextrose broth)

The PDA medium, weighing 39 grammes, was dissolved in a small amount of double distilled water. The pH was adjusted to 7.0±0.2 by adding dilute acid and base. The volume was then raised to 1000ml with double distilled water and the mixture was autoclaved. A solution was prepared by dissolving 20 grammes of glucose and 10 grammes of 80 peptone in a small amount of double distilled water. The pH of the solution was adjusted to 7.0±0.2, and the volume was then raised to 1000 ml by adding more double distilled water. The culture media underwent sterilisation in the autoclave at a pressure of 15 lb for a duration of 15 minutes. The aseptic media was placed onto petri dishes and allowed to harden in the laminar airflow. Wells were created in the petri dishes using a sterile cork borer with an internal diameter of 8 mm. The plates that had been manufactured were utilised to assess the antifungal efficacy.

Antifungal Activities

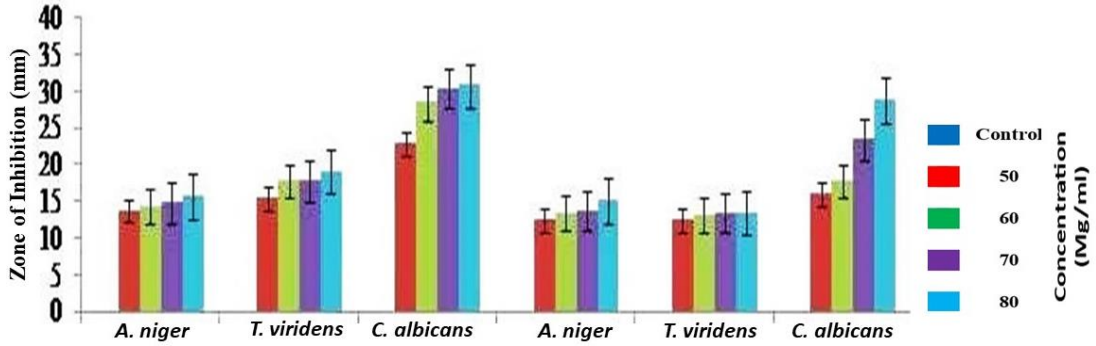
The antifungal properties of the extracts were examined using the agar well diffusion method. The fungus culture was created by transferring a full loop of fungus from potato dextrose agar slants into the sabouraud dextrose broth. The culture was then cultured at a temperature of 27°C for a duration of 72 hours. The newly obtained fungal cultures were utilised to assess their antifungal properties.

The wells were created using a sterile cork borer with an internal diameter of 8 mm. Then, 100 µl of methanol, ethanol, chloroform, and hexane extracts of Ashoka tree were applied to each well at concentrations of 50 mg/ml, 60 mg/ml, 70 mg/ml, and 80 mg/ml. Controls were preserved using appropriate solvents. Fluconazole at a concentration of 50 µg/ml was utilised as the positive control. The test findings were obtained by three replicates and the average values were computed.

Result

Dry Leaves powder Extract	**Inhibition Zone(mm)**					
	Methanol			**Ethanol**		
	A. niger	***T. viridens***	***C. albicans***	***A. niger***	***T. viridens***	***C. albicans***
50mg	13.67±0.57	15.33±0.57	22.67±1	12.33±0.00	12.33±0.57	16±0.57
60mg	14.33±0.57	17.67±0.57	28.33±1.15	13.33±0.57	13±0.57	17.67±0.57
70mg	14.67±0.57	17.67±0.57	30.33±0.57	13.67±0.57	13.33±0.57	23.33±0.00
80mg	15.67±0.00	19±0.57	30.67±057	15±0.00	13.33±0.57	26.67±0.57
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
F value	484.4	940.0	756.4	567.2	514.7	1604

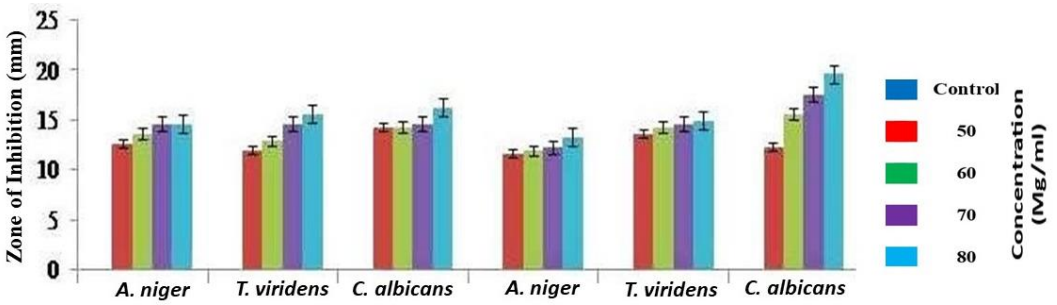
Table 1: Antifungal activity of Plant Leaves Extract of methanol and ethanol



Graph-1 Dry leaves powder extract of methanol and ethanol

Dry Flower Powder Extract Inhibition Zone(mm)						
	Chloroform			**Hexane**		
	A. niger	***T. viridens***	***C. albicans***	***A. niger***	***T. viridens***	***C. albicans***
50mg	12.67±0.57	12±0.00	14.33±0.57	11.67±0.57	12.67±0.57	13.33±0.57
60mg	13.67±0.57	12.33±0.57	14.33±0.57	12±0.00	14.33±0.57	15.67±0.57
70mg	14.67±0.57	15.67±0.57	14.67±0.57	12.33±0.57	14.67±0.57	17.67±0.57
80mg	14.67±0.57	15.67±0.57	16.33±0.57	13.33±0.57	15±0.00	19.67±0.57
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
F value	567.2	347.3	508.4	468.7	627.2	682.8

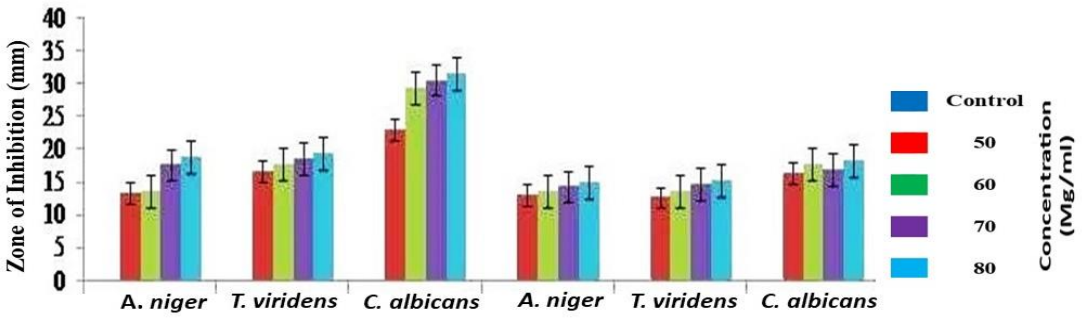
Table 2: Antifungal activity of Dry Flower Powder Extract of chloroform and hexane



Graph-2 Dry Flower Powder Extract of chloroform and hexane

Dry Seed Powder Extract Inhibition Zone(mm)						
	Methanol			**Ethanol**		
	A. niger	***T. viridens***	***C. albicans***	***A. niger***	***T. viridens***	***C. albicans***
50mg	13.33±0.57	16.67±0.00	23±0.00	13±0.57	12.67±0.57	16.33±0.57
60mg	13.67±0.57	17.67±0.57	29.33±0.57	13.67±0.00	13.67±0.57	17.67±0.57
70mg	17.67±0.57	18.67±0.57	30.67±0.57	14.33±0.57	14.67±0.57	17±0.57
80mg	19±0.57	19.33±0.57	31.67±0.57	15±0.57	15.33±0.00	18.33±0.57
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
F value	917.2	747.3	1307	894.5	457.8	909.7

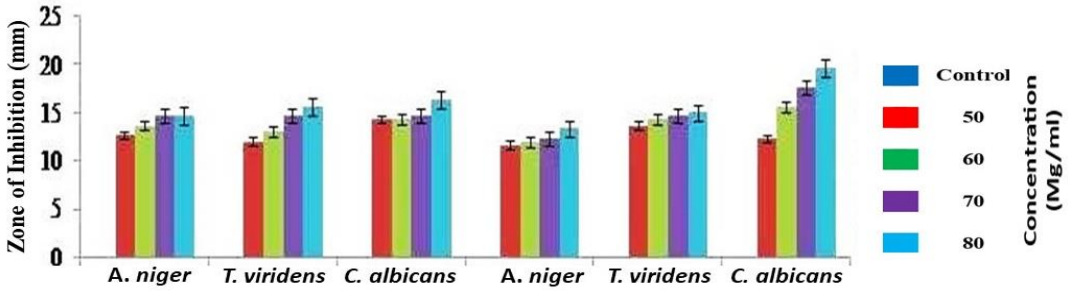
Table 3: Antifungal activity of Dry Seed Powder extract of methanol and ethanol



Graph-3 Dry Seed Powder extract of methanol and ethanol

Dry Bark powder Extract Inhibition Zone(mm)						
	Chloroform			**Hexane**		
	A. niger	***T. viridens***	***C. albicans***	***A. niger***	***T. viridens***	***C. albicans***
50mg	12.67±0.57	12±0.00	14.33±0.57	11.67±0.57	13.67±0.57	12.33±0.57
60mg	13.67±0.57	13±0.57	14.33±0.57	12±0.00	14.33±0.57	15.67±0.57
70mg	14.67±0.57	14.67±0.57	14.67±0.57	12.33±0.57	14.67±0.57	17.67±0.57
80mg	14.67±0.57	15.67±0.57	16.33±0.57	13.33±0.57	15±0.00	19.67±0.57
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
F value	425.9	627.2	508.4	468.7	627.2	682.8

Table 4: Antifungal activity of Dry Bark powder Extract of chloroform and hexane



Graph-4 Dry Bark powder Extract of chloroform and hexane

Discussion

The extract exhibited higher inhibition zones in ethanol than methanol against all microorganisms, with maximum zones of 30.67±0.57 mm and 26.67±0.57 mm, respectively, at 80mg concentration. Ethanol extracts showed a more pronounced inhibitory effect against C. albicans, while methanol extracts were more effective against *A. niger*. Overall, the extract's antimicrobial activity was more pronounced in ethanol solvent.

The dry flower powder extract exhibited significant inhibitory activity against *A. niger, T. viridens,* and *C. albicans*, with increasing inhibition zones at higher concentrations (50-80mg) in both hexane and chloroform solvents. C. albicans was most susceptible, with a maximum inhibition zone of 19.67±0.57 mm at 80mg in chloroform. *A. niger* and *T. viridens* showed similar inhibition patterns, with maximum zones of 16.33±0.57 mm and 15.67±0.57 mm, respectively, at 80mg in hexane.

comparison of the inhibition zones of the dry seed powder extract in methanol and ethanol solvents: The extract exhibited higher inhibition zones in methanol than ethanol against all microorganisms, with maximum zones of 31.67±0.57 mm and 19.33±0.57 mm, respectively, at 80mg concentration. Methanol extracts showed a more pronounced inhibitory effect against *C. albicans*, while ethanol extracts were more effective against *A. niger*. Overall, the extract's antimicrobial activity was more pronounced in methanol solvent.

The extract exhibited similar inhibition zones in both hexane and chloroform solvents, with maximum zones of 19.67±0.57 mm and 16.33±0.57 mm, respectively, at 80mg concentration. However, chloroform extracts showed a slightly higher inhibitory effect against *C. albicans*, while hexane extracts were more effective against *A. niger*. Overall, the extract's antimicrobial activity was comparable in both solvents.

The extracts exhibited varying levels of antimicrobial activity against *A. niger, T. viridens*, and *C. albicans*, with ethanol and methanol solvents showing distinct efficacy profiles. Ethanol extracts were more effective against *C. albicans*, while methanol extracts were more effective against *A. niger*. Overall, the choice of solvent significantly impacted the extract's antimicrobial activity.

Conclusion

In conclusion, the study highlights the significant impact of solvent choice on the antimicrobial activity of the extracts against *A. niger, T. viridens*, and *C. albicans*. Ethanol and methanol solvents exhibited distinct efficacy profiles, with ethanol more effective against *C. albicans* and methanol more effective against *A. niger*. Hexane and chloroform solvents showed comparable antimicrobial activity. The findings underscore the importance of selecting the appropriate solvent for optimal antifungal activity.

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