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STUDY ON THE ANTIFUNGAL PROPERTIES OF SOME SELECTED MEDICINAL PLANTS AGAINST *ASPERGILLUS* SP.

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Abstract–This study investigates the antifungal properties of four important medicinal plants extracts Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Neem (*Azadirachta indica*), and Turmeric (*Curcuma longa*) against two pathogenic fungi, *Aspergillus* and *Penicillium* at different concentrations. These fungi are known to cause a variety of diseases in both humans and crops, leading to significant health and economic concerns. To investigate the antifungal properties of natural plant extract from Neem (*Azadirachta indica)*, Ginger (*Zingiber officinale)*, Garlic (*Allium sativum)* and Turmeric (*Curcuma longa)* against the fungal pathogen *Aspergillus.* The antifungal efficacy of aqueous and ethanolic extracts from ginger, garlic, neem, and turmeric was evaluated using disc diffusion and broth micro dilution methods to determine their minimum inhibitory concentrations (MICs) and zones of inhibition. This study will suggest that these natural substances possess significant antifungal potential, which could be explored for the development of alternative antifungal agents in both agricultural and medicinal applications. Further research is needed to identify the active biochemical compounds responsible for this activity and to assess the safety and effectiveness of these extracts in clinical and environmental settings**.**

INTRODUCTION

The use of plants as a source of medicine dates back to ancient civilizations and continues to be a prominent practice today, especially in the treatment of infectious diseases. Herbal medicine is deeply rooted in the traditions of many cultures where plants have long been utilized to treat various ailments such as malaria, diarrhoea, burns, gonorrhoea, and stomach disorders (Tagoe *et al.*, 2011). The World Health Organization (WHO) reports that approximately 80% of the global population relies on traditional medicine, which often involves plant extracts or their active compounds, as a primary form of healthcare (Nogueira *et al*., 2010). This synergy between plants and their bioactive compounds highlights the significance of ethno pharmacology and the potential for harnessing natural resources in the fight against infectious diseases. Thus, the continued study and use of plant-based medicines are vital for expanding treatment options, especially in areas where modern healthcare may be limited (Gautam *et al*., 2011).

Aspergillus niger belongs to genus *Aspergillu*s and is one of the most commonly occurring fungus that is a member of ascomycetes (Baker, 2006). *Aspergillus niger* is not as dangerous or deadly as other species of *Aspergillu*s, but being opportunistic can definitely cause sickness and allergic reactions (Schuster *et al*., 2002). *Aspergillus niger* is a very thermo-tolerant fungus that can withstand in freezing conditions, wide range of pH and in very hot weather (Pitt and Hocking, 2009). *Aspergillus niger* contains different types of toxins namely malformin C, and ochratoxin A. If there is minute inhalation of *Aspegillus niger* spores then people can be safe and no infection can be caused but continuous exposure of spores can cause serious problems. Otomycosis is a sever type of very painful ear infection of outer ear canal caused by *Aspergillus niger* (Shah *et al*., 2004). Besides the darker side of *Aspergillus niger*, it can produce useful enzymes through the process of fermentation and helpful in production of citric acid, starch processing, baking, brewing and beverage industries, in animal feed and in the paper and pulping industry (Tudzynski *et al*., 2002; Baker, 2006). *Aspergillus niger* has the ability of bioabsorption of silver and lead (Gade *et al*., 2008; Mahmoud, *et al*., 2011; Matrose *et al*., 2021).

Garlic has been widely used in traditional medicine since ancient time, and garlic oil has been shown to have many medicinal effects, such as antitumor genetic, ant carcinogenic, ant atherosclerotic, antithrombotic, Antidiabetic, ant platelet aggregation, anti-inflammatory activity, and etc. (Liu *et al*., 2005). Although there are many studies on the antimicrobial activity of garlic oil (Benkeblia, 2004; Mondali *et al*. 2009, Saradhajyothi and Subbarao, 2011). The *Curcuma* genus has a long history of medicinal applications (Akarchariya *et al*., 2017; Dosoky and Setzer, 2018), being composed of approximately 120 species. Among the *Curcuma* species, *Curcuma longa L*. (*Curcuma*; Turmeric) is the most widely recognized; a cultivated plant, grown in a warm climate, in many regions of the world (Wu, 2015). A multitude of beneficial pharmacological properties have been granted to the Curcuma species, including antiproliferative, antiinflammatory, anticancer, antidiabetic, anti-diarrheal, carminative, diuretic, antirheumatic, antimicrobial, antiviral, antioxidant, larvicidal, insecticidal, antivenomous, and antityrosinase effects, among others (Reanmongkol *et al*., 2006; Lin *et al*., 2010; Angel *et al*., 2014). *Azadirachta indica*, also known as Neem, Nimtree and Indian Lilac is a tree in the mahogany family - Meliaceae. *Azadirachta indica* is commonly used for the treatment of Diabetes and shows the potential role of Antidiabetic activity (Shravan and Kannappan, 2011; Hassan, *et al*., 2010; Kutawa *et al*., 2018, Li *et al*., 2016). Ginger (*Zingiber officinale*) has long been valued in traditional medicine for its diverse health benefits, including its antifungal properties. Key bioactive compounds in ginger, such as gingerol, shogaol, and zingerone, are believed to play a significant role in combating fungal infections. Gingerol, the primary compound in fresh ginger, has been shown to exhibit strong antimicrobial and antifungal effects (Rasooli *et al.,* 2006; Singh and Shukla, 2015; Behiry *et al*., 2022).

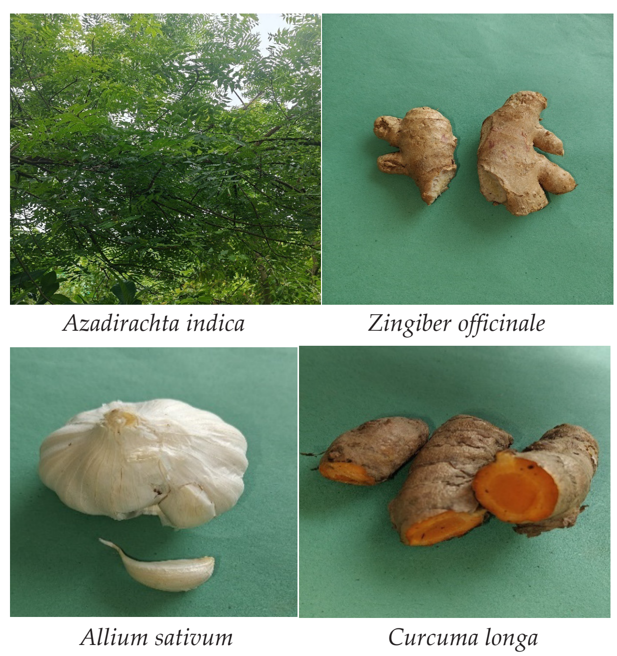
MATERIALS AND METHODS

Collection of Plant Species

Fresh leaves of Neem (*Azadirachta indica*). Rhizomes of ginger (*Zingiber officinale)*, Garlic (*Allium sativum* and Turmeric Rhizomes (*Curcuma longa)*were collected from the university campus ensuring they were free from visible disease, damage, or contamination. The samples were carefully plucked using sterile gloves and stored in clean, sterile polythene bags. *Aspergillus* sp was isolated from contaminated sample Apple and identification was done.

Macroscopic Identification (Colony Morphology)

After the incubation period, the culture plates were observed for the development of fungal colonies. *Aspergillus* colonies were tentatively identified based on their distinctive macroscopic characteristics: Color: Colonies appeared green, black, or brown depending on the species. Texture: Typically powdery, woolly, or velvety. Growth Pattern: Rapidly spreading colonies, sometimes with concentric ring formations. Reverse Side Coloration: The underside of the colony on the agar plate was also observed, which may help differentiate between species. For confirmatory identification, a Lactophenol Cotton Blue (LPCB) mount was prepared for each suspected *Aspergillus* colony. Confirmed *Aspergillus* colonies were sub-cultured onto fresh PDA slants to obtain pure cultures. These were labeled and preserved at 4°C for use in subsequent antifungal activity testing with neem,



Photoplate 1. Photographs showing collected specimens (*Azadirachta indica, Zingiber officinale, Allium sativum, Curcuma longa)*

ginger, garlic, and turmeric extracts (Gaddeyya *et al*., 2012).

Antifungal Testing by Agar well diffusion Method

To evaluate the antifungal activity of medicinal plant extracts, the agar well diffusion method is commonly used. Fresh PDA media was prepared and extract of Neem, Ginger, Garlic and Turmeric at 2%, 3%, 4% were poured into sterile Petri plates. Using a sterile 10ul pipette, *Aspergillus* spore was added on the surface of each agar plate. After inoculation of test fungi the petri plates were incubated at a temperature of 25-30 °C for 48-72 hours. The zone of inhibition were measured in centimeters. The zone of inhibition for each plant extract against *Aspergillus* were compared and were measured. Larger inhibition zones indicate higher antifungal activity (Mirtaghi *et al*., 2016).

RESULTS AND DISCUSSION

In the present day, study to evaluate the antifungal properties of Neem, Ginger, Garlic, and Turmeric against *Aspergillus* species using the culture plate method, different concentrations (2, 3 %, and 4 %) of each plant extract were tested. In all four cases, 2 % of each plant extract showed the most fungal growth of *Aspergillus*, 3 % showed slightly less growth, and 4 % showed the least fungal growth. This indicates that the antifungal activity of Neem, Ginger, Garlic, and Turmeric extracts increases with higher concentrations against *Aspergillus* species (Table 1).

The present study evaluated the antifungal efficacy of Neem, Ginger, Garlic, and Turmeric extracts at varying concentrations (2%, 3%, and 4%) against *Aspergillus* species. The results revealed a consistent trend across all tested plant extracts, increasing concentrations led to a significant reduction in fungal growth (Abu *et al*., 2008; Maj *et al*., 2024). Among the extracts, the 2% concentration exhibited the highest fungal growth, indicating a relatively weak antifungal effect at lower doses. As the concentration increased to 3%, there was a moderate reduction in fungal colony formation, while the 4% concentration demonstrated the least fungal growth, highlighting a dose-dependent antifungal activity (Khan *et al*., 2012; Li *et al*., 2016; Behiry *et al*., 2022). Singh and Shukla (2015) also reported the inhibitory potential of ginger extracts against pathogenic fungi. Benkeblia (2004), who demonstrated that garlic extract has significant antifungal action against *Aspergillus* sp and other filamentous fungi. Huang *et al*. (2011), also emphasized the antifungal properties of curcumin against a range of phytopathogenic fungi (Amenu *et al*., 2024) (Figure 1).

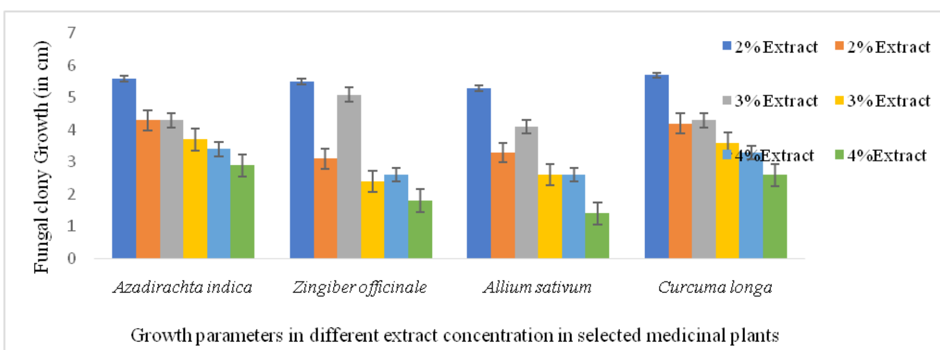


Fig. 1. Variation of fungal growth in each extract (*Azadirachta indica, Zingiber officinale, Allium sativum, Curcuma longa)*

Table 1. Effect of different concentrations of medicinal plant extracts against *Aspergillus* sp.

Plant Extract	2% Extract		3% Extract		4% Extract	
	Length (in cm)	Breadth (in cm)	Length (in cm)	Breadth (in cm)	Length (in cm)	Breadth (in cm)
Azadirachta indica	5.6±0.2	4.3±0.4	4.3 ±0.2	3.7 ±0.3	3.4±0.2	2.9 ±0.2
Zingiber officinale	5.5 ±0.3	3.1±0.3	5.1±0.4	2.4±0.2	2.6 ±0.3	1.8±0.3
Allium sativum	5.3±0.4	3.3±0.2	4.1±0.2	2.6±0.3	2.6±0.4	1.4 ±0.4
Curcuma longa	5.7±0.2	4.2±0.5	4.3 ±0.3	3.6±0.2	3.3±0.5	2.6 ±0.2

The values are the mean of three replicates (n=3), ± indicates standard deviation.

CONCLUSION

The present investigation was aimed at evaluating the antifungal properties of Neem (*Azadirachta indica)*, Ginger (*Zingiber officinale)*, Garlic (*Allium sativum)*, and Turmeric (*Curcuma longa)* extracts against *Aspergillus* species using the culture plate method. Different concentrations of each extract (2 %, 3 %, and 4 %) were tested to assess their ability to inhibit the growth of *Aspergillus*. The results of this study revealed a clear dose-dependent antifungal activity for all four plant extracts, where an increase in the concentration of extracts corresponded to a greater inhibition of fungal growth.Overall, this study clearly demonstrates that the antifungal efficacy of Neem, Ginger, Garlic, and Turmeric extracts against *Aspergillus* species is dose-dependent, with higher concentrations producing greater inhibitory effects on fungal growth. The increase in antifungal activity at 4 % of each extract can be explained by the higher concentration of bioactive compounds, which likely exert stronger antifungal actions through multiple mechanisms such as disrupting fungal cell wall integrity, inhibiting enzyme activity, altering membrane permeability, and interfering with fungal metabolism. These findings are in alignment with several previous research works, which have documented the antimicrobial and antifungal potential of these plant extracts against various fungal species. Overall, the findings confirm that higher concentrations of plant extracts enhance their antifungal efficacy, which could be attributed to the increased availability of bioactive compounds at elevated doses. The study supports the potential use of these natural plant extracts as eco-friendly, cost-effective alternatives to synthetic fungicides for the management of fungal infections caused by *Aspergillus* sp.

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Conflict of Interest – None

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