Antifungal properties of methanolic leaf extract of *Acacia nilotica* against *Aspergillus fumigatus*

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Abstract

The present study was designed to investigate the antifungal potential of methanolic leaf extracts of *Acacia nilotica* extracts against *Aspergillus fumigatus*, a common fungal pathogen known for causing various postharvest diseases. In this study, methanolic leaf extract was prepared using standard extraction methods and its antifungal activity was evaluated through liquid broth assay. For that, four treatments *viz.* 2%, 4%, 6% and 8% of the leaf extract were prepared in 2% malt extract broth. After autoclaving, the fungal suspension was inoculated in the prepared methanolic extract containing medium. After seven days of incubation growth of mycelium (fresh and dry weight) and antioxidant enzyme (peroxidase and catalase) activities were estimated. Results revealed significant ($P \leq 0.05$) antifungal activity of *A. nilotica* methanolic leaf extracts against *A. fumigatus*, with decreasing mycelial growth up to 99% in comparison with control. The results indicated effective inhibition of fungal growth at relatively low concentrations of the leaf extracts. Additionally, the study explored that the enzyme activities of tested fungi was significantly increased by 2 folds as compared to control. Overall, the findings suggest that native *A. nilotica* possesses promising antifungal properties against *A. fumigatus*, highlighting its potential as a natural source of antifungal agents for combating fungal infections.

Keywords: *Acacia nilotica*, Antifungal properties, *Aspergillus fumigatus*, Methanolic leaf extract.

Introduction

In recent years, an increasing interest in investigation of natural sources for novel antifungal agents has been observed possibly due to the emergence of drug-resistant fungal strains and the limitations of existing antifungal drugs. Antifungal agents play a crucial role in combating fungal infections, which pose significant threats to human health, agriculture, and the environment. With the rise of drug-resistant fungal strains and limitations associated with conventional antifungal therapies, there is a pressing need to explore alternative sources of antifungal compounds such as medicinal plants (Cui *et al*., 2022; Javaid *et al.*, 2023).

Among alternate sources, medicinal plants have shown promising potential as a rich reservoir of bioactive compounds with diverse pharmacological activities, including antifungal properties (Khan *et al.*, 2021; Choudhary *et al*., 2022; Ferdosi *et al.*, 2022). *A. nilotica*, commonly known as the gum arabic tree, is a plant species widely distributed in various regions across Africa and Asia. Traditionally, different parts of *A. nilotica* are being used to treat various ailments due to its diverse pharmacological properties. In particular, the leaves of *A. nilotica* have gained attention for their potential therapeutic benefits, including antimicrobial activities. Studies have shown that various extracts and phytochemicals derived from different parts of *A. nilotica* possess significant antifungal activity against a range of fungal pathogens. These include species such as *Candida albicans*, *Aspergillus fumigatus*, *Macrophomina phaseolina* and *Fusarium* spp., which cause opportunistic infections in humans and various diseases in plants (Javaid *et al.*, 2018; Kumar *et al.*, 2024).

A. fumigatus is one of the most clinically relevant fungal pathogens, known to cause a wide range of infections, particularly in immune-compromised individuals. Due to the increasing incidence of aspergillosis and the limited efficacy of current antifungal therapies, there is a pressing need to discover and develop new antifungal agents with improved efficacy and safety profiles (Arastehfar *et al*., 2021). In this study, we aimed to investigate the antifungal properties of methanolic leaf extract of native *A. nilotica* against *A. fumigatus*. Methanol was chosen as the solvent for extraction due to its ability to efficiently extract a wide range of bioactive compounds from plant materials. The choice of *A. fumigatus* as the target pathogen was based on its clinical significance and prevalence in fungal infections.

Materials and Methods

Collection of pathogen

The 1st Fungal Culture Bank of Pakistan provided culture of *A. fumigatus* for this study. The obtained sample was stored at 4 °C after sub-culturing on 2% malt extract agar medium.

Collection of plant leaves

Leaves of *A. nilotica* were collected in February 2023 along the bank of Indus River near Mari Indus, Pakistan. The weather of sampling site was cool and dry during sampling period. Samples were collected from seven sites and combined.

Preparation of plant extracts

The healthy collected leaves were separated and cleaned with tap water followed by drying in sun light for 3 days. Then, 100 mL of methanol was added in 40 g of dried and crushed leaves, and placed at room temperature for 10 days. Thereafter, it was filtered oven-dried at 60 °C for 2 days. Later on, 0.25 mL of dimethyl sulfoxide (DMSO) was added to dissolve it and raised the volume up to 10 mL.

***In vitro* study**

A laboratory experiment was conducted to access the efficacy of selected plant extract against *A. fumigatus*. The formula $C_1V_1 = C_2V_2$ was utilized. Each plant extract was subjected to various dilutions, which were prepared at concentrations 2, 4, and 8%. In this context, C_1 showed the concentration of stock solution while C_2 represented the target concentration for the diluted solution. Likewise, V_2 showed the volume required for the final solutions of different concentrations. Subsequently, the diluted plant extract was combined with malt extract (2%) and autoclaved for 45 min at 121 °C and a pressure of 103 kPa. The growth medium was then inoculated with 0.5 µL of the fungal spore suspension and incubated at a temperature of 35±2 °C. Each treatment had three replicates.

Mycelial growth analysis

After one week of incubation, *A. fumigatus* the fresh and dry weight was calculated. To determine the dry weight of fresh fungal mycelium, a drying process is typically employed where the moisture contents was removed leaving behind the dry biomass, it was oven dried for over-night at 60 °C.

Biochemical attributes

After seven days of incubation, the total protein content (Lowry, 1951) and antioxidant enzyme activities like peroxidase (Kumar and Khan, 1982) and catalase (Mayer *et al.*, 1965) activities were estimated in homogenized mycelial mixture with chilled sodium phosphate buffer having a concentration of 100 mM and pH 6.8.

Statistical analysis

Statistical analysis of the data was conducted by using LSD Test. All calculations were performed by using the software Statistix 8.1.

Results

Growth parameter

Fresh weight of *A. fumigatus* was found to be significantly ($P \leq 0.05$) decreased by 27 to 88% with the increasing concentration of methanolic leaf extract of *A. nilotica* (2 to 8%) in comparison with control. Likewise, the dry weight was also significantly declined by 51 to 99% due to 2 to 8% concentrations of methanolic leaf extract of *A. nilotica* as compared to control (Fig. 1).

Total protein content

The results revealed that the total protein content of *A. fumigatus* when treated with the methanolic leaf extract of *A. nilotica* at different concentrations significantly increased up to ~ 2 folds over control (Fig. 2A).

Catalase activity

It was noticed that the catalase activity of the tested fungus when treated with *A. nilotica* methanolic extract, was significantly ($P \leq 0.05$) enhanced by 2 folds in comparison with control (Fig. 2B).

Peroxidase activity

The results depicted that the peroxidase activity in the fresh mycelial mass of *A. fumigatus* was significantly ($P \leq 0.05$) higher by ~ 2 folds due to application of 2 to 8% of the leaf extract in comparison with control (Fig. 2C).

Discussion

Methanolic leaf extract of *A. nilotica* was taken at different levels *i.e.* 2%, 4%, 6% and 8% to control the growth of *A. fumigatus.* The result revealed that the treatment with tested methanolic leaf extract significantly) declined the fresh and dry weight upto 99% in comparison with control. Several studies have demonstrated the significant antifungal activity of *A. nilotica* extracts against various fungal species. For instance, Alyousef (2021) conducted a study evaluating the antifungal potential of *A. nilotica* bark extract against clinical isolates of *Candida* species and found promising inhibitory effects. Similarly, Guevara-Lora *et al.* (2020) reported the effectiveness of *A. nilotica* leaf extracts against human pathogenic fungi. Moreover, Jose and Sinha (2016) found that the bark and leaf methanolic extracts of *A. nilotica* as compared to the root extract, demonstrated considerable antifungal effectiveness against *Aspergillus flavus*. Rahman *et al*. 2014 showed that methanolic leaf extract of *A. nilotica* from different sources exhibited antifungal action against *A. flavus in vitro*.

The modulation of enzyme activity in fungi by *A. nilotica* agents may involve multiple mechanisms. Sarkiyayi and Abubakar *et al.* (2018) analyzed the biochemical attributes in methanolic leaf extract of *A. nilotica* that revealed the presence of cardiac glycoside, alkaloids, steroids, tannins, saponins, flavonoids, anthraquinones and terpenoids. These phytochemicals have the ability to work against different diseases. These tests will facilitate the isolation of chemical compounds with pharmacological properties consequently, the leaves of *A. nilotica* harbor diverse secondary metabolites,

notably alkaloids which exhibit various pharmacological effectslike analgesic and anti-malarial activity, thereby holding significant promise in phytomedicine. Tannin on the other hand, might be attribute to preventing heart disease displaying antioxidant activity, or scavenging free radicals (Ali *et al*., 2017).

It was also noticed in the present study that the total protein content and antioxidant enzymes (catalase and peroxidase) activities were significantly increased by 2 folds in *A. fumigatus* when treated with *A. nilotica* at 2 to 8% concentrations. Antioxidant enzymes play a vital role in fungal defense against oxidative stress induced by various environmental factors, including exposure to natural compounds like those found in *A. nilotica*. Recent studies have provided evidence suggesting that *A. nilotica* extracts can modulate the activities of antioxidant enzymes in fungi. For example, Almanaa *et al.* (2022) investigated the effect of *A. nilotica* leaf extracts on antioxidant enzyme activities in *A. flavus*. They observed a significant increase in the activities of these enzymes in response to *A. nilotica* treatment, indicating a potential adaptive response to oxidative stress. The modulation of antioxidant enzyme activities in fungi by *A. nilotica* extracts may involve several mechanisms. One possible mechanism is the activation of signaling pathways involved in oxidative stress response. *A. nilotica* extracts contain bioactive compounds such as flavonoids and polyphenols, which can stimulate the expression and activity of antioxidant enzymes through transcriptional regulation or post-translational modifications (Gazzar and Ismail, 2020). Moreover, they also reported that *A. nilotica* extracts may directly scavenge reactive oxygen species (ROS), thereby lowering oxidative stress and relieving the burden on antioxidant defense systems. Certain phytochemicals present in *A. nilotica* extracts such as tannins and alkaloids, possess antioxidant properties and can neutralize ROS directly or indirectly, thereby enhancing the efficiency of antioxidant enzymes in fungi.

Conclusion

The results support the notion that native Pakistani *A. nilotica* could serve as a valuable source for developing novel antifungal therapies for combating *A. fumigatus* infections. Further research is warranted to explore the efficacy and safety of *A. nilotica* extracts in clinical settings and to uncover the specific mechanisms of action underlying their antifungal properties.

Author's contributions

SA conceived the idea, carried out statistical analysis and supervised the work. S conducted experiment. NJ wrote the first draft of manuscript while AS carried out the final editing of the manuscript.

Conflict of interests

Authors declare no conflict of interest.

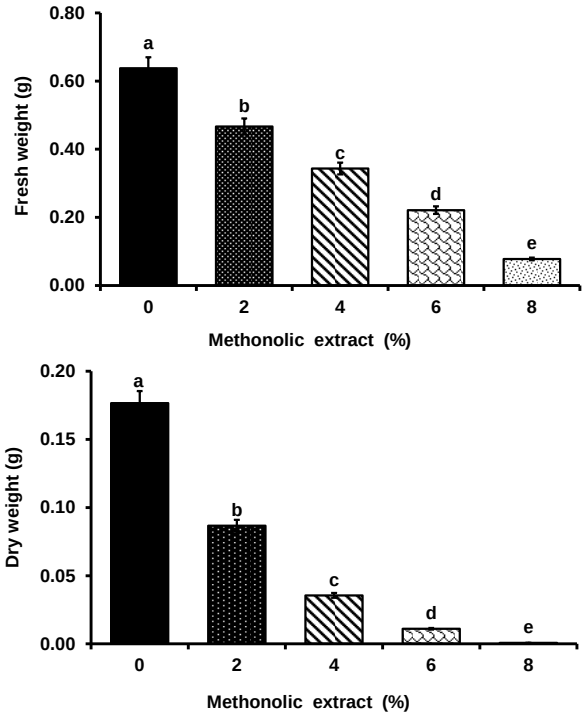

Fig. 1: Effect of methanolic leaf extract of *Acacia nilotica* on the fresh and dry weight of *Aspergillus fumigatus* after seven days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by LSD test at $P \leq 0.05$.

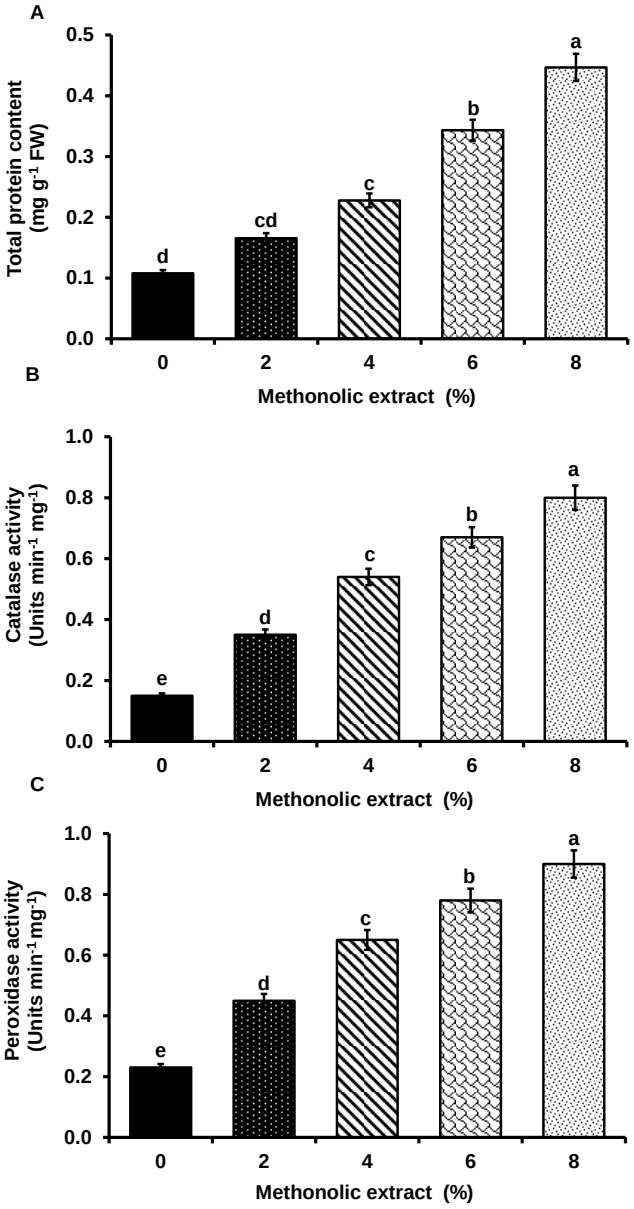

Fig. 2: Effect of *Acacia nilotica* methanolic leaf extract on total protein content (A), catalase activity (B), and peroxidase activity of *Aspergillus fumigatus* after seven days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by LSD test at $P \leq 0.05$.

References

Ali S, Ali K, Hussain Z, Khan MS, Khan W M, Wali, S, Shuaib M. 2017. Phytochemical screening and antimicrobial activity of selected medicinal plant species. *Pure Appl. Biol.*, **6**: 418-425.

Almanaa TN, Rabie G, El-Mekkawy RM, Yassin MA, Saleh N, EL-Gazzar N, 2022. Antioxidant, antimicrobial and antiproliferative activities of fungal metabolite produced by *Aspergillus flavus* on in vitro study. *Food Sci. Technol. Campinas.*, **42**: e01421.

Alyousef AA, 2021. Antifungal activity and mechanism of action of different parts of *Myrtus communis* Growing in Saudi Arabia against *Candida* spp. *J. Nanomater.*, **2021**: 3484125.

Arastehfar A, Carvalho A, Houbraken J, Lombardi L, Garcia-Rubio R, Jenks JD, Hoenigl M, 2021. *Aspergillus fumigatus* and aspergillosis: From basics to clinics. *Stud. Mycol.*, **100**: 100115.

Choudhary M, Kumar V, Naik B, Verma A, Saris

PEJ, Kumar V, Gupta S, 2022. Antifungal metabolites, their novel sources, and targets to combat drug resistance. *Front. Microbiol.*, **13**: 1061603.

Cui, X, Wang, L, Lü, Y, Yue C, 2022. Development and research progress of anti-drug resistant fungal drugs. *J. Infect. Public Health*, **5**: 986-1000.

El-Gazzar N, Ismail AM, 2020. The potential use of titanium, silver and selenium nanoparticles in controlling leaf blight of tomato caused by *Alternaria alternata*. *Biocatal. Agric. Biotechnol.*, **27**: 101708.

Ferdosi MFH, Khan IH, Javaid A, Saeed HM, Butt I, Munir A, 2021. GC-MS analysis and bioactive components of flowers of *Bergenia ciliata*, a weed of rock crevices in Pakistan. *Pak. J. Weed Sci. Res.*, **27**: 527-535.

Ferdosi MFH, Ahmed H, Khan IH, Javaid A, 2022. Fungicidal potential of flower extract of *Cassia fistula* against *Macrophomina phaseolina* and *Sclerotium rolfsii. J. Anim. Plant Sci.*, **32**: 1028-1034.

Guevara-Lora I, Bras G, Karkowska-Kuleta J, González-González M, Ceballos K, Sidlo W, Rapala-Kozik M, 2020. Plant-derived substances in the fight against infections Caused by *Candida* species. *Int. J. Mol. Sci.*, **21**: 6131.

Javaid A, Ali A, Khan IH, Ferdosi MFH, 2023. Leaves of *Chenopodium album* as source of natural fungicides against *Sclerotium rolfsii*. *Arab. J. Chem.*, **16**: 104677.

Javaid A, Kanwal A, Shoaib A, 2018. Effect of *Trichoderma harzianum* and dry leaves of *Acacia nilotica* subsp. *indica* on growth of mash bean in *Macrophomina phaseolina* contaminated soil. *Int. J. Biol. Biotechnol.*, **15**: 535-540.

Khan IH, Javaid A, Naqvi SF, 2021. Molecular characterization of *Penicillium expansum* isolated from grapes and its management by leaf extract of *Chenopodium murale. Int. J. Phytopathol.*, **10**: 29-35.

Kumar A, Kumar S, Singh KM, Tiwari SK, 2024. A comprehensive review on the chemical composition and pharmacological activities of *Acacia arabica*. *Intelligent Pharm.*, https://doi.org/10.1016/j.ipha.2024.01.008

Kumar KB, Khan PA, 1982. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv PR 202) leaves during senescence. *Indian J. Exp. Biol.*, **20**: 412-416.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.

Mayer AM, Harel E, Shaul RB, 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry*, **5**: 783-789.

Rahman A, Shakoor A, Zaib G, Mumtaz AS, Ihtesham Y, Napar AA, 2014. Comparative antimicrobial activity of *Acacia nilotica* L. leaves extracts against pathogenic bacteria and fungi. *J. Med. Plant Res.*, **8**: 975-982.

Sarkiyayi S, Abubakar MA, 2018. Effect of ethanol leaf extract of *Acacia nilotica* on phenyl hydrazine induced anemia in rats. *IOSR J. Pharm. Biol. Sci.3*, **13**: 1-10.