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Phytochemical Profiling and Antifungal Potential of Young Stem Extracts of *Moringa oleifera* Against Plant Pathogenic Fungi

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Abstract

Plants are natural repositories of bioactive metabolites that defend against pathogens and offer sustainable alternatives to chemical fungicides. This study assessed the phytochemical composition and antifungal efficacy of young stem extracts of *Moringa oleifera*. Soxhlet extraction with ethanol yielded crude extracts, which were screened for phytochemicals using standard assays. Alkaloids, carbohydrates, phenolic compounds, tannins, phytosterols, and carboxylic acids were detected, whereas flavonoids and triterpenoids were absent. Antifungal activity was evaluated against *Fusarium oxysporum*, *Alternaria solani*, and *Colletotrichum capsici* using poisoned food and agar well diffusion methods. Growth inhibition was dose-dependent, with maximum inhibition (72.4%) against *C. capsici* at 300 mg/mL. These findings highlight the potential of *M. oleifera* stems as eco-friendly antifungal resources.

Keywords: *Moringa oleifera*, phytochemistry, antifungal activity, secondary metabolites, natural fungicides

Introduction

Fungal pathogens are responsible for significant agricultural losses globally, affecting staple and horticultural crops (Pareek *et al.,* 2023). Synthetic fungicides are widely used for management, but challenges such as resistance development, chemical residues, and ecological toxicity have prompted a search for sustainable, plant-derived alternatives (Punia and Singh, 2018).

Plants produce an array of secondary metabolites such as alkaloids, phenolics, tannins, and sterols, which function as natural antimicrobials. For example, phenolic compounds interfere with fungal enzymatic pathways, while tannins disrupt protein structures in pathogen cell walls (Abhang *et al.,* 2024).

Moringa oleifera, a multipurpose plant native to South Asia and Africa, is known as the "miracle tree" due to its nutritional, medicinal, and environmental importance (Gautam *et al.,* 2023). While its leaves and seeds are well studied for antimicrobial activity, the phytochemistry and antifungal potential of young stems remain underexplored (Nweke, 2016). Given that pathogens such as *Fusarium oxysporum*, *Alternaria solani*, and

Colletotrichum capsici cause devastating diseases like wilt, early blight, and anthracnose respectively, this study aims to investigate the phytochemical profile and antifungal activity of *M. oleifera* young stem extracts.

Literature Review

Recent studies confirm the antimicrobial potential of *M. oleifera*. Leaf extracts have been reported to suppress *Fusarium* spp. and *Rhizoctonia solani* (Majumder *et al.,* 2024), attributed to their high phenolic and flavonoid content. Bark extracts exhibited inhibitory effects on *F. oxysporum* when tested by poisoned food technique, confirming the antifungal potential of stem tissues (Punia and Singh, 2018).

Broader reviews also underline the rich phytochemistry of *M. oleifera*, including alkaloids, sterols, saponins, and tannins (Pareek *et al.,* 2023). Comparative studies across leaves, seeds, and bark show that solvent polarity influences metabolite detection and antifungal activity (Abhang *et al.,* 2024). Recent GC-MS profiling studies further identified fatty acids, sterols, and phenolic derivatives in leaves, suggesting a similar but underexplored potential in stems (Mamgain *et al.,* 2024).

Despite these findings, very few investigations have focused exclusively on the young stems, justifying the present research to assess their phytochemical profile and antifungal efficacy.

Materials and Methods

Plant Collection and Extraction

Young stems of *M. oleifera* were collected from authenticated plants in Jaipur, India. The plant material was washed, shade-dried, powdered, and subjected to Soxhlet extraction using ethanol for 8 h. The extract was concentrated with a rotary evaporator and stored at 4 °C.

Fig: (i) Young stem material

Fig: (ii) Soxhlet extraction

Fig: (iii) Soxhlet extration of extract

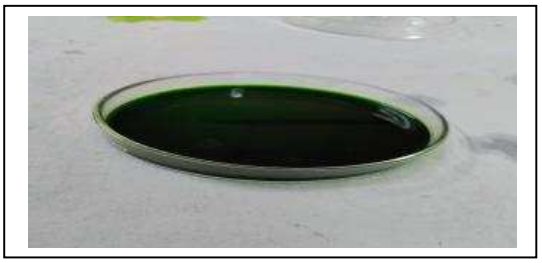

Fig: (iv) Final liquid extract

Phytochemical Screening

Phytochemical screening followed standard protocols (Nweke, 2016; Abhang *et al.,* 2024). Tests included Mayer's, Wagner's, and Dragendorff's for alkaloids; Benedict's and Iodine for carbohydrates; ferric chloride for phenolics; gelatin and lead acetate for tannins; sodium hydroxide and Shinoda for flavonoids; Salkowski's and Liebermann–Burchard for sterols and triterpenoids; and effervescence with sodium bicarbonate for carboxylic acids.

Antifungal Assays

Antifungal activity was tested against *F. oxysporum*, *A. solani*, and *C. capsici* using two methods:

Poisoned Food Technique – PDA medium amended with extracts at 100, 200, and 300 mg/mL. Fungal plugs placed centrally, incubated at 28 °C, and inhibition percentage calculated (Gakuubi *et al.,* 2017).

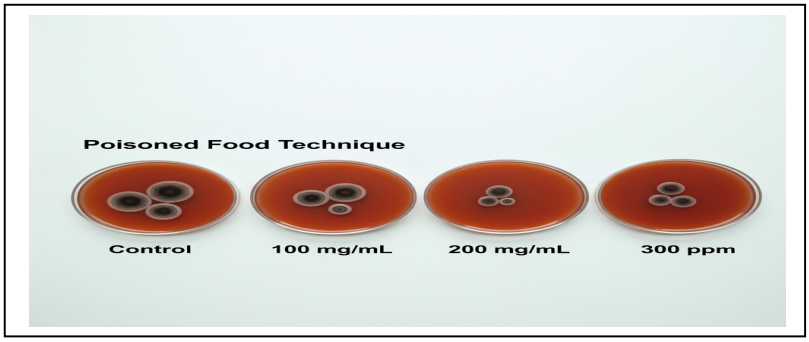

Fig: (v) Poisoned Food Technique for assessing antifungal activity

Agar Well Diffusion Method – PDA plates inoculated with fungal suspensions; wells filled with extract; inhibition zones measured after 72 h (Veeraswamy *et al.*, 2022).

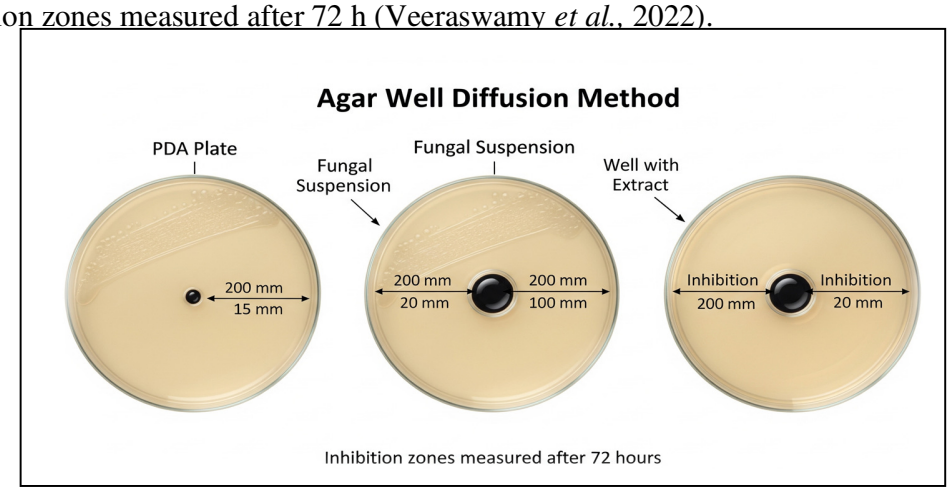

Fig: (vi) Inhibition zones measured after 72 hours

All assays were performed in triplicate, with results analyzed by one-way ANOVA ($p \leq 0.05$).

Results

Phytochemical Profile

The phytochemical screening demonstrated the presence of alkaloids, carbohydrates, phenolic compounds, tannins, phytosterols, and carboxylic acids, while flavonoids and triterpenoids were absent. These findings suggest that young stems harbor secondary metabolites associated with antifungal activity.

Table 1: Phytochemical composition of *Moringa oleifera* young stem extract

Phytochemical class	Tests used	Observation	Result
Alkaloids	Mayer's, Wagner's, Dragendorff's	Cream/orange/reddish precipitate	Present
Carbohydrates	Benedict's, Iodine	Brick-red/blue-black coloration	Present
Phenolics	Ferric chloride, Folin–Ciocalteu	Blue-green coloration	Present
Tannins	Gelatin, Lead acetate	White precipitate	Present
Flavonoids	Sodium hydroxide, Shinoda	No color change	Absent
Phytosterols	Salkowski's, Liebermann–Burchard	Reddish-brown ring	Present
Triterpenoids	Liebermann–Burchard	No yellow ring	Absent
Carboxylic acids	Effervescence with $NaHCO_3$	Bubbling observed	Present

Fig: (vii) Detection and test result images of phytochemicals

Antifungal Efficacy

The antifungal assays revealed a clear dose-dependent inhibition. At 300 mg/mL, inhibition was 68.9% for *F. oxysporum*, 70.3% for *A. solani*, and 72.4% for *C. capsici*. Similar trends were confirmed by the agar well diffusion method, with inhibition zones increasing with extract concentration.

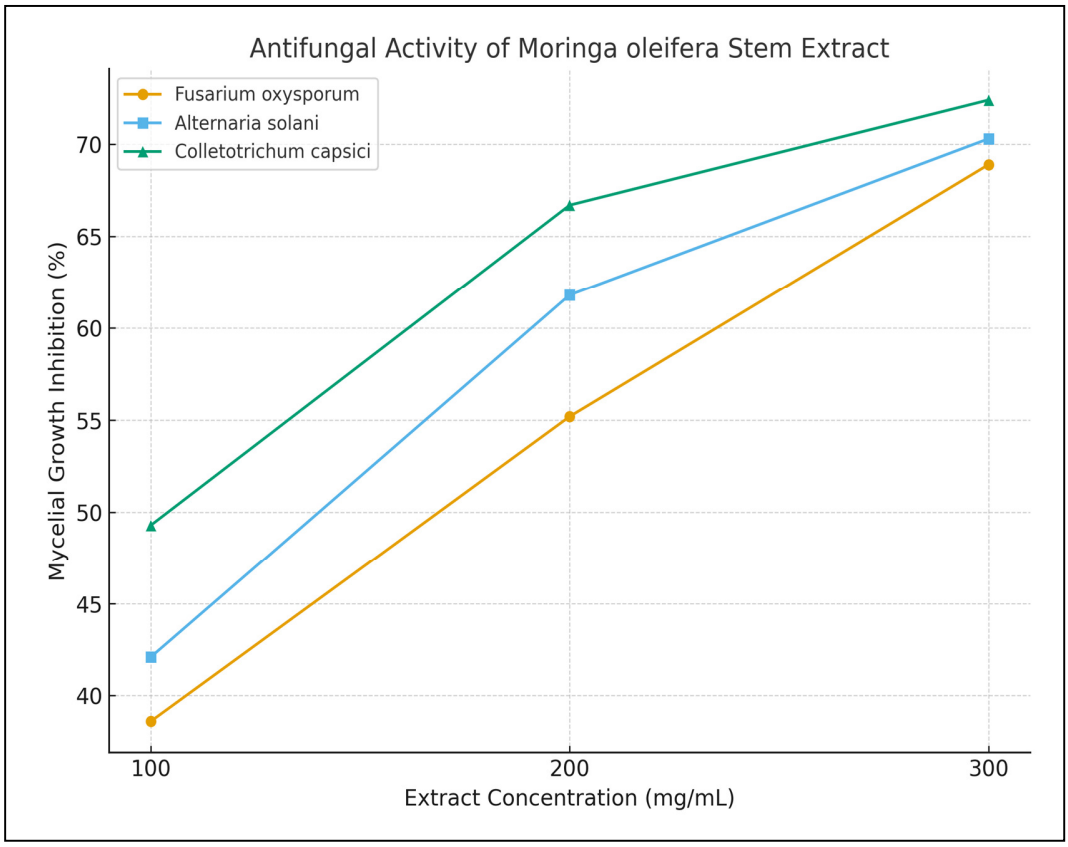

Fig: (viii) Percentage inhibition of fungal pathogens at different extract concentrations (100–300 mg/mL).

Discussion

The phytochemical results align with earlier reports that *M. oleifera* tissues contain alkaloids, phenolics, tannins, and sterols, which are known contributors to antifungal action (Pareek *et al.,* 2023; Gautam *et al.,* 2023). The absence of flavonoids in stems, unlike leaves, suggests tissue-specific metabolite allocation (Abhang *et al.,* 2024).

The antifungal activity demonstrated here is consistent with prior findings that *M. oleifera* extracts suppress phytopathogens such as *F. oxysporum* and *Rhizoctonia solani* (Punia and Singh, 2018; Majumder *et al.,* 2024). The highest inhibition against *C. capsici* indicates a strong potential for use in chili anthracnose management. This supports the hypothesis that stem-derived metabolites contribute to natural plant defense mechanisms.

Conclusion

The ethanolic extract of *M. oleifera* young stems was found to contain multiple bioactive phytochemicals and demonstrated significant antifungal activity against *F. oxysporum*, *A. solani*, and *C. capsici*. These findings establish the potential of young stems as a source of natural antifungal compounds and encourage further research on compound isolation, MIC/MFC determination, and field trials.

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