ID: 649

Antifungal Activity of Methanolic Leaf Extract of *Gmelina arborea* Roxb. ex Sm. Against *Fusarium oxysporum* Schltdl Causing Damping -Off Disease

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Abstract

Fusarium oxysporum, the causal agent of damping-off disease, is a destructive soilborne pathogen that significantly reduces seedling establishment and crop yield. Although synthetic fungicides remain effective in controlling this pathogen, their continuous and excessive use has led to challenges such as resistance development, environmental pollution, and health hazards. In this study, the antifungal activity of methanolic leaf extract of *Gmelina arborea* Roxb. ex Sm. was evaluated against *F. oxysporum* causing damping off disease. Fresh leaves of *Gmelina arborea* were collected, authenticated, dried, and extracted using 70% methanol by maceration. Pure strains of *Fusarium oxysporum* were obtained and identified from Mycology Laboratory, Department of Crop protection, Ahmadu Bello University, Zaria. Phytochemical screening of *Gmelina arborea* leaf extract revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, and cardiac glycosides, while anthraquinones were absent. Quantitative analysis showed high levels of flavonoids (125.41 mg/g) and phenols (124.98 mg/g), both known for their antimicrobial activity. The antifungal effect of the extract was tested in vitro by incorporating graded concentrations (5–25 mg/mL) into potato dextrose agar and monitoring mycelial growth inhibition. Results demonstrated a concentration-dependent effect, with maximum inhibition (68.3%) observed at 20 mg/mL. Interestingly, inhibition decreased at 25 mg/mL (54.9%), possibly due to antagonistic interactions at higher concentrations. These findings demonstrate that *G. arborea* possesses promising antifungal potential against *F. oxysporum*. The presence of bioactive phytochemicals, particularly flavonoids and phenols, likely accounts for the observed inhibitory effects. Therefore, the extract could serve as a natural alternative to synthetic fungicides in the management of damping-off disease.

Key Words: *Antagonistic ; fungicides; maceration ; Phytochemical screening; soil borne pathogen*

Introduction

Fungal pathogens are among the most significant biological threats to global food production (Anand and Rajeshkumar, 2022). One such pathogen is *Fusarium oxysporum*, a soilborne ascomycete fungus responsible for a variety of plant diseases, including damping-off, root rot, wilt, and vascular discoloration in crops such as tomato (*Solanum lycopersicum*), okra (*Abelmoschus esculentus*), beans (*Phaseolus spp.*), spinach (*Spinacia oleracea*), and many others (Haruna et al., 2024). Among these diseases, damping-off is particularly notorious in nurseries and early crop stages, where it causes the rapid wilting and collapse of seedlings, leading to poor crop establishment and severe yield losses (Delai et al., 2024). A wide range of crops are susceptible to damping-off disease, especially during the seedling stage include tomato, cabbage (*Brassica oleracea*) (Pagoch et al., 2015) lettuce (*Lactuca sativa*), carrot (*Daucus carota*), pepper (*Capsicum spp.*), onion (*Allium cepa*), cucumber (*Cucumis sativus*), okra, spinach, melon (*Cucumis melo*), eggplant (*Solanum melongena*), maize (*Zea mays*), and soybean (*Glycine max*) (Agrios, 2005; Lamichhane *et al.*, 2017). These crops are especially vulnerable under conditions of high humidity, waterlogged soil, and poor aeration, which favour the growth and spread of damping-off pathogens such as *Fusarium spp.*, *Pythium spp.*, and *Rhizoctonia solani* (Lamichhane*et al.*, 2017).

Damping-off caused by *Fusarium oxysporum* is prevalent in warm and moist soil environments, conditions common in tropical and subtropical regions. The pathogen can survive in soil and plant debris for long periods as chlamydospores, which are highly resistant to environmental stress (Admas and Kebede, 2024).). When seedlings are infected with *F. oxysporum*, the fungus colonizes the root cortex and vascular tissues, causing root decay, vascular browning, and wilting that often result in seedling death within days of germination (Sanogo and Ji, 2021).

Damping-off disease signific-antly impacts crop production worldwide, especially in nurseries and early crop stages, by causing rapid seedling decay and death, leading to poor crop establishment and reduced yields. Globally, this disease is responsible for substantial economic losses in vegetable, cereal, and ornamental crop production, particularly under high humidity and poor soil drainage conditions (Lamichhane *et al.*, 2017). In Nigeria, damping-off is a major constraint in both subsistence and commercial farming, affecting crops such as tomato, pepper, okra, and leafy vegetables. Its prevalence is heightened by the country's tropical climate, characterized by warm temperatures and frequent rainfall, which create ideal conditions for damping-off pathogens like *Fusarium*

oxysporum, *Pythium spp.*, and *Rhizoctonia solani* (Pathak, (2023); Aigbe *et al.*, 2017). Farmers often suffer seedling losses in nurseries, resulting in increased costs for replanting and delayed crop cycles, thereby threatening food security and income.

Chemical fungicides such as benomyl and carbendazim have been traditionally employed to manage *F. oxysporum*-induced damping-off disease. However, over-reliance on these chemicals has resulted in resistance development in pathogen populations, environmental degradation, and toxicity to non-target organisms (Yoon *et al.*, 2022). There is an increasing call for sustainable and eco-friendly alternatives to synthetic fungicides.

Plant-based extracts have emerged as promising alternatives because they are biodegradable, cost-effective, and environmentally safe. Medicinal plants, in particular, are rich in bioactive compounds such as flavonoids, tannins, alkaloids, and phenols, which have shown strong antimicrobial properties (Omotunde *et al.*, 2025). One such plant is *Gmelina arborea* is a fast-growing tree belonging to the family Lamiaceae. Traditionally used in herbal medicine across Africa and Asia, *Gmelina arborea* has been reported to possess antifungal, antibacterial, antioxidant, and anti-inflammatory properties (Nwachukwu *et al.*, 2022; Aliyu *et al.*, 2023). While preliminary studies have confirmed the antimicrobial potential of *G. arborea*, its antifungal efficacy against *Fusarium oxysporum*, particularly in relation to damping-off disease, remains underexplored.

This research is aim to fill that gap by providing scientific evidence on the efficacy of methanolic *G. arborea* leaf extract as a natural alternative to synthetic fungicides against *Fusarium oxysporum* causing damping off disease of crops.

Materials and Methods

Study Area

The study was carried out in the Department of Pharmacognosy and Drug Development, and Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Zaria is located between latitude 11º09'N, longitude 7º42'E at an altitude of 686 meters above sea level.

Sample Collection of Gmelina arborea Plant and the Fungal Pathogen

The leaves of *Gmelina arborea* were collected from the Botanical garden of Ahmadu Bello University, Zaria, and authenticated in the herbarium of the Department of Botany, Ahmadu Bello University, Zaria. Pure strains of *Fusarium oxysporum*were obtained and identified from Mycology Laboratory, Department of Crop protection, Ahmadu Bello University, Zaria.

Preparation of Extract of Leaves of Gmelina Arborea

This was done using maceration method (Ayoola *et al.*, 2008). 56g of the sample was weighed using an electronic balance (Atom model), ground into powder with a laboratory blender (Chins, 2.00 RPM), and dissolved in 70% methanol in an aspirator bottle. After 72 hours of extraction, the supernatant was decanted into a beaker, then transferred to a rotary evaporator (Haulolph) to remove the methanol solvent, leaving the extracted oil. The purified oil was collected and stored in a sealed bottle for further analysis.

Quantitative and Qualitative Phytochemical Screening of Gmelina arborea

Determination of Total Phenolic Content (TPC)

Estimation of total phenol content was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using Gallic acid as the standard .The total phenol was determined with the help of standard cure prepared from pure phenolic standard (Gallic acid) (Alhakmani *et al*., 2013; Nikolaeva et al., 2022).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric assay (Farooq *et al.*,2021).

Determination of Total Alkaloid Content (TAC)

Total Alkaloid Content was quantified by spectrophotometric method. This method was based on the reaction between alkaloid and bromocresol green (BCG). (Pourahadi, *et al*., 2019).

Determination of Tannin Content

The tannins were determined by Folin - Ciocalteu method. The tannin content was expressed in terms of mg of GAE /g of extract (Koleva *et al*., 2021).

Determination of Saponins Content

Total saponins was determined in accordance to the method of Ajiboye *et al.* (2020).

Determination of Anthraquinone Content

Anthraquinone content was determined by the method of Enin *et al.* (2021).

Determination of Cardiac Glycoside Content

The Cardiac Glycoside was determined by the method of Onyekachi (2021).

Determination of Terpenoid Content

The terpenoid content was determined by the method of Kajal and Singh, (2019).

Determination of Steroid Content

Steroid content was estimated using the method as described by Malik (2017).

Determination of Total Carbohydrate Content

The phenol–sulfuric acid was determined by the method of Nielsen (2024).

Evaluating The Antifungal Activity of Gmelina arborea Extracts Against Fusarium oxysporum

A pure culture of *Fusarium oxysporum* was obtained from the Mycology Laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria. The culture was sub-cultured on fresh Potato Dextrose Agar (PDA) and incubated at 27°C for 7 days to obtain actively growing mycelia suitable for inoculation. The already-prepared methanolic extract of *Gmelina arborea* was incorporated into molten PDA medium at different concentrations (5mg/mL, 10 mg/mL, 15 mg/mL, 20mg/mL and 25 mg/mL). Appropriate volumes of the extract were mixed with PDA cooled to 45–50°C, ensuring even distribution. 25 mL of each amended medium was poured into sterile Petri dishes. After the media solidified, each Petri dish was inoculated at the center with a 2 mm mycelial disc taken from the edge of a 7-day-old culture of *P. colocasiae*. The plates were sealed with parafilm to prevent contamination and incubated at 27°C for 7 days.

Data Collection

Fungal growth was monitored daily by measuring colony diameter in two perpendicular directions. The extent of inhibition was determined by comparing treated plates with control plates. The percentage of mycelial growth inhibition was calculated using the formula:

Percent Inhibition (%) = C−T/C × 100

Where:

- **C** = colony diameter in control plate (mm)
- **T** = colony diameter in treatment plate (mm)

Experimental Design And Statistical Analysis

One way ANOVA was used to determine the effect of different concentrations of the extract on the growth inhibition of *Fusarium oxysporum*. Where significant difference is observed between the treatments. Means were separated using least significant difference (LSD). All analysis were performed using SPSS at $p<0.005$ level of significance.

Results

Qualitative Phytochemical Analysis Screening

The qualitative phytochemical analysis of *Gmelina arobrea* extracts shows the presence of nine phytochemicals including, Alkaloids, Tannins, Saponins, Flavonoids, Terpenoids, Steroids, Phenols, Cardiac Glycosides and Carbohydrates. The results also show that Anthraquinones are absent in *Gmelina arborea*extracts (Table 1).

Table 1: Phytochemical compounds present in the methanolic leaf extract of *Gmelina arborea*

S/No	*Phytoconstituents*	*Test*	*Inference*
1	Alkaloids	Dragendorff test	+
2	Cardiac Glycosides	Keller-Kiliani test	+
3	Saponins	Frothing test	+
4	Phenolic compounds	Lead acetate test	+
5	Tannins	Ferric Chloride test	+
6	Steroids	Salkowiski test	+
7	Carbohydrates	Molisch test	+
8	Flavonoids	Shinoda test	+
9	Terpeniods	Liebermann Burchard test	+
10	Anthraquinones	Bontragers test	-

Keys; + PRESENT, ABSENT

Quantitative Phytochemical Screening of the Extract

Quantitative phytochemical screening showed the quantity of phytochemicals associated with the methanolic solution of *Gmelina arborea.* It was found out the methanolic extract has the highest phytochemical content in Flavonoids 125.41mg/g.

Table 2: Quantitative phytochemical compounds present in the methanolic leaf extract of *Gmelina arborea*

S/N	***CONSTITUENT***	***Gmelina arborea(mg/g)***
1	Alkaloids	12.02
2	Flavonoids	125.41
3	Saponins	22.21
4	Phenolic compounds	124.98
5	Tannins	85.87

Table 3: Effect of different concentrations of methanolic leaf extract of *Gmelina arborea* on percentage mycelial growth inhibition of *Fusarium oxysporum*

Concentration (mg/ml)	***Inhibition (%)***
Control	26.3 ± 12.2c
5	46.8 ± 19.6b
10	49.6 ± 6.3b
15	53.6 ± 10.7ba
20	68.3 ± 17.3a
25	54.9 ± 2.6ba

Data are expressed as mean ± standard deviation. Means that share the same subscript letter are not significantly different from each other, as determined by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at $p > 0.05$.

Discussion

The present study revealed that *Gmelina arborea* leaves contain a wide range of phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and cardiac glycosides, while anthraquinones were absent. The high levels of flavonoids and phenolic compounds are of particular significance, as these metabolites are well known for their antioxidant and antifungal properties. Their abundance may be explained by the plant's defensive metabolic strategies, since secondary metabolites are often synthesized in response to environmental stress, herbivory, or pathogen attack. Methanol, the extraction solvent used in this study, is particularly effective at solubilizing flavonoids and phenolics, which may account for their prominence in the extract.

These findings corroborates with Musa (2022), who reported the presence of alkaloids, flavonoids, tannins, and saponins in *G. arborea* leaves, similarly, Seepe *et al.* (2021) confirmed the presence of flavonoids, phenolic compounds, and cardiac glycosides. However, the absence of anthraquinones contrasts with Singh *et al.* (2020), who reported their presence in the bark, suggesting differences due to plant part, solvent, or ecological factors.

The antifungal assays further supports the role of these phytochemicals, with *G. arborea* extracts producing concentration-dependent inhibition of *Fusarium oxysporum*. Inhibition increased steadily with increased extract concentration and peaked at 20 mg/ml, but declined at 25 mg/ml, showing that higher concentrations did not always enhance activity. Similarly, non-linear responses have been reported by Seepe *et al.*, (2021; Musa (2022) in plant-based antifungal studies, where inhibition decreases at high concentrations due to antagonistic phytochemical interactions or reduced solubility of bioactive compounds. Recent studies have reported comparable patterns. Gutierrez et al., (2017), demonstrated that high concentrations of plant compounds initially suppressed bacterial growth but subsequently permitted recovery. Similarly, investigations on *Gmelina arborea* have shown that its extracts exert strong antibacterial effects at lower concentrations, yet the inhibitory activity tends to stabilize as concentrations increase (Singh and Mijakovic, 2022).

This study found that *Gmelina arborea* has strong inhibitory effects that increase with concentration before stabilizing, showing its potential as a natural source of therapeutic compounds. These effects highlight the importance of its phytochemicals, which act together in complex ways, and support its role as a promising alternative to synthetic drugs for managing microbial activity and related health issues.

The significant inhibitory effects of *G. arborea* align with reports of other flavonoid- and tannin-rich plant extracts showing strong antifungal activity. The findings of this study are consistent with those of Adedire *et al.* (2021), who examined the biocontrol potential of *Gmelina arborea* wood shavings against cassava root-rot pathogens, including *Fusarium oxysporum*. Their results revealed that aqueous extracts of the plant significantly

suppressed fungal mycelial growth, achieving inhibition rates exceeding 80% at effective concentrations. This aligns with the present study, which also demonstrated strong inhibitory activity of *G. arborea* extracts against *Fusarium* oxysporum. Although different plant parts and extract types were used, both studies collectively reinforce the antifungal potential of *G. arborea* and validate its role as a natural source of bioactive compounds for managing fungal pathogens as reported by (Adedire et al., 2023).

The study indicates that *Gmelina arborea* leaf extracts possess strong antifungal activity against *Fusarium oxysporum*. This effect is likely due to the presence of phenolics and saponins, which interfere with fungal metabolism, inhibit spore germination, and weaken cell wall proteins. Similar antifungal effects were observed in *Azadirachta indica* (Ali *et al*., 2025), *Allium sativum* (Hari *et al*., 2025), *Glycyrrhiza glabra* (Redondo-Blanco et al.,2020), *Medicago sativa* all of which demonstrate that phytochemical content is central to inhibiting fungal growth, supporting the findings of Chen *et al.* (2025).

This study also indicates that *Gmelina arborea* leaf extracts demonstrate strong antifungal activity against *Fusarium oxysporum*, which is likely due to the synergistic action of flavonoids, phenols, tannins, and saponins, which work together to inhibit fungal growth. Previous studies support this finding, Shitu and Aliyu (2024) linking these metabolites in ethanolic leaf extracts to strong antimicrobial effects.

Conclusion

The study demonstrated that the methanolic leaf extract of *Gmelina arborea* possesses significant antifungal activity against *Fusarium oxysporum*. Phytochemical analysis revealed high levels of flavonoids (125.41 mg/g) and phenolics (124.98 mg/g), which likely contributed to the inhibitory effect.

The extract achieved the highest growth inhibition of 68.3% at 20 mg/mL, followed by 54.9% at 25 mg/mL, while the control recorded the lowest inhibition of 26.3%. Notably, the inhibition percentage did not consistently increase with concentration, as inhibition at 25 mg/mL was lower than at 20 mg/mL. This suggests that beyond a certain threshold, higher concentrations may not proportionally enhance antifungal activity, possibly due to saturation or antagonistic effects within the extract.

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