

Investigating the Use of Vegetable Waste Pepper Pedicles to Combat Food Spoilage by Fungi

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Abstract

This study investigated the antifungal activity of ethanolic extracts of pepper pedicles (*Capsicum annuum*) against food spoilage fungi, *Aspergillus* spp. and *Penicillium* spp., as well as their potential application in preserving tomato fruits. The extracts were prepared using ethanol and tested at various concentrations (500–3.91 mg mL⁻¹) using the agar well diffusion method, while ketoconazole served as the standard antifungal control at concentrations of 500–25 mg mL⁻¹. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined to assess the efficacy of the extract. The results revealed that the ethanolic extract exhibited concentration-dependent inhibition on both fungal isolates, with *Penicillium* spp. being more sensitive. The highest inhibition zone (20 mm) was observed at 500 mg mL⁻¹, while activity declined at lower concentrations. MIC and MFC values for *Penicillium* spp. were 6.25 mg mL⁻¹ and 12.5 mg mL⁻¹, respectively, whereas *Aspergillus* spp. recorded 25 mg mL⁻¹ and 50 mg mL⁻¹. In the tomato preservation assay, the 125 mg mL⁻¹ extract delayed spoilage up to seven days, compared to the control, which spoiled within three days. These findings indicate that pepper pedicle extracts possess strong antifungal potential, comparable in effect to standard antifungal agents. The presence of bioactive compounds such as capsaicinoids and phenolics likely contributed to the inhibitory activity observed. Therefore, the study concludes that pepper pedicle extract could serve as a natural, eco-friendly antifungal agent for food preservation and spoilage prevention, promoting sustainable utilization of agricultural by-products.

Keywords: Pepper pedicles, antifungal activity, *Aspergillus* spp., *Penicillium* spp., MIC, MFC, food spoilage

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1. Introduction

Food spoilage is a major global challenge that threatens food security and safety, with fungi being among the primary contributors. Fungal contamination leads to visible decay, discoloration, unpleasant odors, and the production of harmful mycotoxins such as aflatoxins and ochratoxins, which pose serious health risks (Smith, 2023; Anonymous, 2023). Globally, it is estimated that approximately 25% of food crops are affected by fungal growth at some stage in the food supply chain, from production to storage (Anonymous, 2023). To mitigate such losses, natural, plant-based antifungal agents have attracted considerable attention. Spices and herbs, including oregano, garlic, and cinnamon, have demonstrated efficacy in extending shelf life and inhibiting fungal growth due to bioactive secondary metabolites such as phenolics and essential oils, which disrupt fungal membranes, inhibit spore germination, and reduce toxin production (Lee et al., 2018; Johnson and Patel, 2020). Peppers (*Capsicum* spp.) are widely recognized for their rich composition of bioactive compounds, particularly capsaicinoids and phenolics, which have demonstrated antifungal properties (Sepúlveda et al., 2024). Capsaicin, responsible for the characteristic heat in chili peppers, has been shown to disrupt fungal cell structures, inhibit ergosterol biosynthesis, and reduce spore formation. Previous studies have reported that aqueous extracts from various *Capsicum* varieties exert fungistatic effects against *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus arrhizus*, and *Alternaria* spp. (Sepúlveda et al., 2024). However, research has primarily focused on the edible portions of peppers, such as the fruit pulp, seeds, or skin, while vegetable wastes like pedicles (stalks) have largely been ignored. Pepper pedicles are likely to contain similar phytochemicals as the fruit and represent an underutilized resource. Studies on other plant wastes, such as banana and onion peels, have demonstrated promising antifungal activity (Ahmed and Hassan, 2020), suggesting that pepper pedicles warrant further investigation as sustainable antifungal agents. Despite the

recognized antifungal potential of plant extracts, the use of pepper pedicles remains largely unexplored. Common spoilage fungi, including *Aspergillus*, *Fusarium*, and *Penicillium* species, continue to threaten food safety by causing significant post-harvest losses and mycotoxin contamination (Phoku et al., 2017; Negedu et al., 2018). Although some natural antifungal agents have been studied in food systems (Zhang et al., 2023), pepper pedicle extracts have received minimal attention, and their effectiveness compared to other plant-based antifungal agents is not well understood. Therefore, investigating the phytochemical composition and antifungal properties of pepper pedicles is both scientifically and practically relevant. This study aims to evaluate the potential of pepper pedicles as a natural, eco-friendly bio-preservative, which could contribute to reducing food spoilage, valorizing agricultural waste, and promoting sustainable food processing.

2. Material and Methods

2.1. Study area

The study was conducted at the Microbiology and Biochemistry Laboratory of Umaru Musa Yar'adua University, Katsina, Nigeria.

2.2. Sample collection and preparation

Fresh pepper pedicles were collected from local farms in Zango Local Government Area, Katsina State, representing commonly discarded agricultural waste (Charyangi Manatunga, 2024). The pedicles were washed under running tap water to remove debris and foreign materials (Senanayake, 2024a), then air-dried at room temperature for 5–7 days to reduce moisture content and inhibit microbial growth (Rodriguez and Raghavan, 2021). The dried samples were ground into a fine powder using a mortar and pestle and stored in clean, airtight containers at room temperature until extraction (Manatunga, 2024; Rodriguez and Raghavan, 2021).

2.3. Extraction of bioactive compounds

Powdered pepper pedicles were accurately weighed and prepared in triplicate for extraction. Ethanol was used as the solvent at a ratio of 1:10 (w v⁻¹) due to its efficiency in dissolving bioactive compounds such as capsaicinoids and phenolics (Costa et al., 2022; Yasin et al., 2023). Samples were soaked in ethanol and agitated on a rotary shaker at room temperature for 48 hours. The mixture was filtered through Whatman No.1 filter paper, and the extraction process was repeated twice to maximize yield (Lee et al., 2021; Zhang et al., 2023). The filtrates were concentrated using a rotary evaporator at 40 °C under reduced pressure, transferred into amber-colored bottles, and stored at 4 °C until further analysis.

2.4. Phytochemical analysis

The extracts were screened for the presence of major bioactive compounds following standard procedures. Alkaloids were detected using Mayer's and Wagner's reagents, saponins by frothing tests, cardiac glycosides via the Keller-Killiani test, flavonoids using NaOH and HCl, steroids with acetic anhydride and H₂SO₄, and terpenes/terpenoids through chloroform-sulfuric acid reactions. Positive color changes or precipitate formation indicated the presence of the respective compounds.

2.5. Antifungal activity assays

2.5.1. Fungal cultures

Food spoilage fungi, including *Aspergillus niger* and *Penicillium* spp., were obtained from the Microbiology Laboratory of Umaru Musa Yar'adua University. Cultures were grown on Potato Dextrose Agar (PDA) plates at ~25 °C for 5–7 days. Spores were harvested in sterile distilled water, and suspensions were adjusted to $\sim 1 \times 10^6$ spores mL⁻¹ using a hemocytometer.

2.5.2. Disc diffusion assay

Sterile filter paper discs (6 mm) were soaked in extract concentrations of 25, 50, and 75 mg mL⁻¹. Fungal suspensions were spread evenly on PDA plates, and discs containing

extracts, a standard antifungal agent (positive control), or solvent only (negative control) were placed on the plates. Plates were incubated at 25 °C for 72 hours, and zones of inhibition were measured in millimeters to assess antifungal activity (Smith et al., 2022; Zhang et al., 2023).

2.5.3. Minimum inhibitory and fungicidal concentrations

MIC and MFC were determined using the broth dilution method (Ado, 2017). Stock solutions (100 mg mL⁻¹) were serially diluted to 50, 25, 12.5, and 6.25 mg mL⁻¹ and added to Sabouraud Dextrose Broth containing fungal inoculum ($\sim 10^6$ CFU mL⁻¹). Tubes were incubated at 28 ± 2 °C for 48–72 hours. MIC was the lowest concentration that inhibited visible growth, while MFC was the lowest concentration with no fungal growth upon subculture onto Sabouraud Dextrose Agar. *Penicillium* spp. exhibited MIC and MFC of 6.25 mg mL⁻¹ and 12.5 mg mL⁻¹, respectively, whereas *Aspergillus* spp. required 25 mg mL⁻¹ and 50 mg mL⁻¹, indicating higher susceptibility of *Penicillium* spp.

2.5.4. Effect on food spoilage

Fresh tomato fruits were treated with varying concentrations of pepper pedicle extract, with ethanol removed using a rotary evaporator to ensure safety. Untreated tomatoes served as controls. All samples were stored at room temperature and monitored daily for fungal growth and spoilage. The time taken for visible spoilage was recorded to evaluate the protective effect of the extracts (Johnson and Patel, 2020; Sepúlveda et al., 2024).

3. Results

Ethanollic extraction of pepper pedicles yielded a thick reddish-brown extract, and phytochemical screening revealed the presence of flavonoids, capsaicin, alkaloids, tannins, and saponins, while glycosides were absent, indicating the presence of bioactive compounds with potential antifungal and preservative properties (Table 1). The extract demonstrated concentration-dependent

antifungal activity against *Penicillium* spp. and *Aspergillus* spp. using the disc diffusion method. Maximum inhibition was observed at 100 mg mL⁻¹, with *Penicillium* spp. showing greater sensitivity (16.5 mm) compared to *Aspergillus* spp. (9.5 mm), and no inhibition was recorded at concentrations below 6.25 mg mL⁻¹ (Tables 2 and 3). The standard antifungal drug ketoconazole consistently exhibited higher inhibitory activity than the extract at all concentrations tested. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays further confirmed

that *Penicillium* spp. was more sensitive than *Aspergillus* spp., with MIC values of 6.25 mg mL⁻¹ and 25 mg mL⁻¹ and MFC values of 12.5 mg mL⁻¹ and 50 mg mL⁻¹, respectively (Table 3). The extract also delayed fungal spoilage of tomato fruits in a concentration-dependent manner. Fruits treated with 125 mg/mL remained fresh until day six, whereas those treated with 62.5 mg mL⁻¹ began to spoil on day five, while untreated controls showed visible spoilage by day three, demonstrating the protective effect of the extract against fungal spoilage (Table 4).

Table 1. Phytochemical composition of ethanolic extract of pepper pedicles

Phytochemical component	Observation
Flavonoids	+ (Present)
Capsaicin	+ (Present)
Alkaloids	+ (Present)
Tannins	+ (Present)
Saponins	+ (Present)
Glycosides	– (Absent)

Table 2. Zones of Inhibition (mm) of pepper pedicle extract against selected food spoilage fungi

Concentration (mg mL ⁻¹)	100	50	25	12.5	6.25	3.11	1.62	0.81
<i>Penicillium</i> spp.	16.5 (VSA)	11 (SA)	10 (SA)	10 (SA)	7.5 (MA)	0 (NA)	0 (NA)	0 (NA)
<i>Aspergillus</i> spp.	9.5 (SA)	7.5 (MA)	6 (MA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)	0 (NA)

Table 3. Zones of inhibition (mm) of ketoconazole against selected food spoilage fungi

Concentration (mg mL ⁻¹)	500	250	125	25
<i>Penicillium</i> spp.	20 (VSA)	17(VSA)	17 (VSA)	17 (VSA)
<i>Aspergillus</i> spp.	20 (VSA)	16 (VSA)	13 (SA)	10 (SA)

Key: VSA – Very Strong Activity, SA – Strong Activity, MA – Moderate Activity, NA – No Activity

Table 4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethanolic extract of pepper pedicles against test fungi

Test Organism	MIC (mg mL ⁻¹)	MFC (mg mL ⁻¹)
<i>Penicillium</i> spp.	6.25	12.5
<i>Aspergillus</i> spp.	25	50

Table 5. Daily observation of fungal spoilage in tomato fruits treated with pepper pedicle extracts

Day	125 mg mL ⁻¹	62.5 mg mL ⁻¹	Control (0 mg mL ⁻¹)
1	No spoilage	No spoilage	No spoilage
2	No spoilage	No spoilage	No spoilage
3	No spoilage	No spoilage	Spoilage observed
4	No spoilage	No spoilage	Spoilage progressed
5	No spoilage	Spoilage observed	Fully spoiled
6	No spoilage	Spoilage progressed	Fully spoiled
7	Spoilage observed	Fully spoiled	Fully spoiled

4. Discussions

This study investigated the antifungal activity of ethanolic extracts of pepper pedicles (*Capsicum annuum* L.) against *Penicillium* spp. and *Aspergillus* spp., evaluating their potential for reducing food spoilage. Disc diffusion assays were employed to assess antifungal potency, while MIC and MFC tests established inhibitory thresholds, and a seven-day tomato storage assay evaluated real-world preservation efficacy. This approach allowed correlation between laboratory inhibition and practical food preservation, providing both scientific and applied relevance. Phytochemical analysis of the extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and capsaicin, compounds widely recognized for their antimicrobial properties, while glycosides were absent (Table 1). These results align with previous reports indicating that pepper by-products retain bioactive metabolites suitable for food and pharmaceutical applications (Anaya-Esparza et al., 2021; Sanatombi, 2025). Capsaicinoids, in particular, have been shown to inhibit fungal growth by disrupting cell wall integrity (Costa et al., 2022), suggesting that these compounds contributed substantially to the observed antifungal effects. Antifungal screening revealed that *Penicillium* spp. was more susceptible than *Aspergillus* spp., with inhibition zones of 16.5 mm and 9.5 mm, respectively, at 100 mg/mL (Tables 2. and 3). This observation is consistent with previous findings indicating that *Penicillium* species are more readily inhibited by *Capsicum* extracts due to weaker enzymatic defenses, whereas *Aspergillus* spp. exhibits partial resistance through thick cell walls and detoxification mechanisms (Phoku et al., 2017; Negedu et al., 2018). The antifungal effect of the extract was concentration-dependent, confirming that higher concentrations enhance inhibition, as reported in other studies on plant bio-preservatives (Lee et al., 2021; Uchegbu et al., 2023). Comparison with the standard antifungal drug ketoconazole showed that although the extract produced slightly smaller inhibition zones (16.5–10 mm vs. 20–17 mm), it still demonstrated clear antifungal activity.

This aligns with reports that natural extracts generally yield moderate inhibition compared to synthetic agents but are safer and environmentally friendly (Ahmed and Hassan, 2020). MIC and MFC results further confirmed the efficacy of the extract, with lower values for *Penicillium* spp. (6.25 mg/mL and 12.5 mg/mL) than for *Aspergillus* spp. (25 mg/mL and 50 mg/mL) (Table 3.3), corroborating previous studies on *Capsicum* extracts and plant polyphenols (Sepúlveda et al., 2024; Zhang et al., 2023). The tomato preservation assay demonstrated that the extract effectively delayed spoilage in a dose-dependent manner, with 125 mg/mL-treated fruits remaining fresh until day six, 62.5 mg/mL-treated fruits spoiling on day five, and untreated controls spoiling by day three (Table 3.4). These results support the practical applicability of the extract as a bio-preservative and are consistent with studies demonstrating delayed spoilage using plant-derived compounds (Lee et al., 2018; Jahun et al., 2021). The antifungal effect is likely due to the disruption of fungal membrane integrity and inhibition of oxidative enzymes, which prolongs fruit shelf life (Smith, et al., 2022; Manatunga, 2024). The strong antifungal performance observed is likely a result of synergistic interactions among multiple phytochemicals, enhancing microbial suppression compared with individual compounds (Senanayake, 2024b; Bekavac et al., 2025). This highlights the advantage of using natural plant extracts, which act on multiple targets and reduce the likelihood of resistance development. From an agricultural perspective, valorizing pepper pedicles, an abundant processing waste, into antifungal extracts presents a sustainable strategy for postharvest management and income generation in regions such as Nigeria's Northern Guinea Savannah (Zakari et al., 2024). Overall, the findings demonstrate that pepper pedicle extracts offer a dual benefit: reducing agricultural waste and providing a natural, eco-friendly alternative to synthetic antifungals. The results support the global trend toward replacing synthetic preservatives with safe, health-conscious alternatives (Costa et al., 2022; Johnson and Patel, 2020) and align

with WHO recommendations for natural interventions to manage mycotoxin contamination (WHO, 2023). Further studies on optimization, stability, and scale-up could establish pepper pedicle extracts as practical bio-preservatives for industrial and household use.

5. Conclusion

The results of this study demonstrate that ethanolic extracts of pepper pedicles (*Capsicum annuum* L.) exhibit significant antifungal activity against *Penicillium* spp. and *Aspergillus* spp., two major spoilage organisms of perishable foods. The presence of bioactive phytochemicals, including alkaloids, flavonoids, tannins, saponins, and capsaicin, contributed to the observed inhibitory effects, confirming the potential of pepper pedicles as a natural source of antifungal compounds. The extract's performance in disc diffusion assays, along with favorable MIC and MFC values, indicates both fungistatic and fungicidal properties, while its ability to delay spoilage in tomato fruits for up to seven days highlights its practical application as a bio-preservative. This study also underscores the economic and environmental relevance of valorizing pepper pedicles, a common agricultural waste, for antifungal purposes. The findings align with previous reports supporting plant-based preservatives as safer and eco-friendly alternatives to synthetic fungicides. By reducing post-harvest losses and minimizing chemical exposure in food systems, pepper pedicle extract offers a dual advantage for food preservation and agricultural sustainability. Therefore, ethanolic extracts of *Capsicum annuum* pedicles represent an effective, affordable, and environmentally friendly antifungal agent, with further research on purification, formulation, and large-scale application recommended to enhance industrial feasibility.

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Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

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