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Antifungal Potential of Indian Plant Extracts Against *Aspergillus niger* Causing Post-Harvest Onion Rot: A Phytochemical Approach.

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ABSTRACT

Post-harvest losses are a significant concern in agricultural economies, particularly in developing nations, where climatic conditions, improper storage, and inadequate handling often result in substantial spoilage. Despite the efficacy of chemical fungicides, the need for safe, sustainable, and cost-effective antifungal strategies has renewed interest in phytochemical-based biocontrol agents. This study evaluates the *in vitro* antifungal activity of four Indian medicinal plant extracts of *Withania somnifera, Piper betle, Cinnamomum verum,* and *Lawsonia inermis* against *Aspergillus niger,* a primary agent of black mold rot in onions (*Allium cepa]*. Extracts were prepared using acetone, methanol, and distilled water. Phytochemical screening, determination of extraction yield, and antifungal testing via agar well diffusion were conducted. *Piper betel* extracts, particularly in acetone, exhibited maximum inhibition (14 mm). The methanol extract of *L. inermis* (12 mm) and the acetone extract of *C. verum* (12 mm) also demonstrated moderate activity against standard antifungal drugs. The findings highlight the potential of plant-derived extracts, especially *Piper betel*, as effective antifungal agents for integrated post-harvest management in agriculture.

Keywords: Aspergillus niger, Onion black mold, Indian medicinal plants, Phytochemicals, Antifungal activity, Solvent extraction, Biocontrol agents, Allium cepa.

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INTRODUCTION

Post-harvest losses are a significant concern in agricultural economies, particularly in developing nations, where climatic conditions, improper storage, and inadequate handling often result in substantial spoilage. It is estimated that nearly one-third of the food produced globally is lost each year, with post-harvest diseases being a key contributor [1]. Among these losses, those occurring in vegetables like *Allium cepa* [onion] are notably severe due to the high perishability of the crop [2].

Onions are cultivated extensively across the globe and hold commercial, culinary, and medicinal significance[3,4]. However, during storage, onions are highly prone to microbial infections, especially fungal diseases. Among the fungal pathogens, *Aspergillus niger* is a predominant species responsible for black mold rot—a post-harvest disease marked by softening and black sporulation on the onion surface [5]. The conditions favoring its growth include elevated moisture, temperature, and damaged tissue, all of which are common during transit and bulk storage[6]. The infection not only reduces the aesthetic and market value of the crop but may also pose a health risk due to potential mycotoxin production [7].

Traditionally, synthetic fungicides such as carbendazim and mancozeb are used to mitigate post-harvest fungal infections [8]. However, the overuse of these chemical agents has led to serious consequences, including the development of resistant fungal strains, accumulation of toxic residues in food and the environment, and disruption of non-target organisms [9–11]. Furthermore, consumer demand is shifting toward organic and chemical-free produce, encouraging researchers to explore safer and more sustainable solutions.

Plants have evolved a rich arsenal of defense mechanisms, many of which are chemical in nature. These include secondary metabolites like alkaloids, flavonoids, phenolics, tannins, and coumarins, which have demonstrated antimicrobial activity in several in vitro studies [12]. Such plant-derived bioactive compounds offer a viable alternative to synthetic fungicides due to their low toxicity, biodegradability, and broad-spectrum antimicrobial effects [13]. The approach aligns with the principles of ethnopharmacology, which seeks to validate traditional medicinal knowledge using modern techniques.

In this context, several Indian medicinal plants with known antimicrobial and antioxidant properties have gained research attention. *Piper betle* (betel), *Withania somnifera* (Ashwagandha), *Cinnamomum verum* (true cinnamon), and *Lawsonia inermis* (henna) are among the most traditionally valued herbs for their therapeutic properties. Previous studies have indicated the presence of bioactive compounds in these plants capable of disrupting microbial cell membranes, interfering with spore germination, and inducing oxidative damage to fungal cells [14–16].

This study focuses on the in vitro evaluation of the antifungal efficacy of leaf extracts from four Indian medicinal plants, prepared in three different solvents like acetone, methanol, and distilled water against *Aspergillus niger* isolated from post-harvest onions. The primary objective is to assess their comparative potential as natural fungicides that could be integrated into sustainable post-harvest management strategies.

MATERIALS AND METHODS

Sample Collection and Isolation of *Aspergillus niger*

Spoiled *Allium cepa* [onion] bulbs showing typical symptoms of black mold rot were collected from markets in Pune, Maharashtra, India. The infected tissue was excised using a sterile scalpel and inoculated into 0.9% normal saline to dislodge surface spores. A loopful of the suspension was streaked onto sterile potato dextrose agar (PDA) plates and incubated at 29 ± 1 °C for 3–5 days. Fungal colonies showing black conidial heads were sub-cultured and identified as *Aspergillus niger* based on colony morphology and microscopic features.

Preparation of Plant Extracts

The collected leaves of *Piper betel, Withania somnifera, Cinnamomum verum*, and *Lawsonia inermis* were washed with distilled water, shade-dried at room temperature for seven days, and pulverized using a laboratory grinder. For extraction, 10 g of each powdered sample was macerated in 100 mL of acetone,

methanol, or distilled water for 72 hours with occasional shaking. Extracts were filtered through Whatman No. 1 filter paper and air-dried at room temperature to obtain a dried residue.

Each dried extract was weighed, and a primary stock solution was prepared by dissolving 5 mg of extract in 5 mL of its respective solvent to obtain a 1 mg/mL stock concentration. From this, a unified working concentration of 0.25 mg/mL was freshly prepared for all plant extracts before testing.

Phytochemical Screening

Qualitative analysis was conducted to determine the presence of major secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, and coumarins using standard procedures[17]. All tests were performed in triplicates.

- **Alkaloids (Mayer's Test):** 1 mL of extract was acidified with dilute HCl and treated with Mayer's reagent. Formation of a creamy white precipitate indicated the presence of alkaloids.
- **Flavonoids (Alkaline Reagent Test):** 1 mL of extract was treated with 2% NaOH. A yellow coloration that disappeared with the addition of dilute HCl confirmed flavonoids.
- **Phenolics (Ferric Chloride Test):** 1 mL of extract was mixed with 5% ferric chloride. Blue, green, or black coloration indicated phenolic compounds.
- **Tannins (Lead Acetate Test):** 1 mL of extract was treated with 1% lead acetate. Precipitate formation indicated tannins.
- **Coumarins (NaOH Test):** 1 mL of extract was shaken with 10% NaOH solution. Yellow coloration confirmed coumarins.

Determination of Extraction Yield

The extraction yield [%] was calculated using the formula:

$$\text{Percentage Yield [\%]} = \frac{\text{Dry weight of extract} \times 100}{\text{Dry weight of plant material}}$$

This was performed for all plant-solvent combinations.[18]

***In Vitro* Antifungal Assay**

Antifungal activity was evaluated by the agar well diffusion method. PDA plates were inoculated with 100 µL of *Aspergillus niger* spore suspension [10^6 spores/mL] and spread uniformly. Wells of 4 mm diameter were punched and loaded with 80 µL of the 0.25 mg/mL working solutions of each plant extract. As per Wang et al.,2024 [19].

A positive control was included using the standard antifungal drug fluconazole at a concentration of 0.25 mg/mL. Solvent-only wells [acetone, methanol, and distilled water] served as negative controls. Plates were incubated at 29 ± 1 °C for 72 hours. Zones of inhibition were measured in millimetres using a Vernier calliper.

RESULTS AND DISCUSSIONS

The percentage yield of crude extracts varied depending on both plant species and the solvent used. As shown in Table 1, the highest yield was recorded for *Piper betle* aqueous extract [Pb-Aq, 3.375%], followed by *Cinnamomum verum* methanol [Cv-MeOH, 3.10%] and *C. verum* aqueous extract [Cv-Aq, 3.225%]. In contrast, the lowest yield was observed in the acetone extract of *Withania somnifera* [Ws-Ace, 2.025%]. Overall, aqueous and methanolic extracts generally exhibited slightly higher yields than acetone extracts.

Despite the higher percentage yield of aqueous extracts, their biological efficacy was limited, suggesting that yield alone is not a direct indicator of bioactivity. Instead, the solubility of bioactive compounds in polar organic solvents like methanol and acetone played a more decisive role in antifungal performance [20].

Qualitative phytochemical analysis of selected medicinal plant extracts revealed the presence of several secondary metabolites known for their antimicrobial activity. Flavonoids, phenolics, tannins, and coumarins were detected predominantly in the acetone and methanolic extracts of *Piper betle*, *Cinnamomum verum*, and *Lawsonia inermis*. In contrast, aqueous extracts showed minimal or no detectable levels of these compounds [As seen in Table 2]. This suggests that solvent polarity played a crucial role in extracting bioactive constituents, which may directly influence the observed antifungal efficacy [21,22].

Flavonoids and phenolics, in particular, are reported to inhibit fungal growth by disrupting cell wall synthesis and inducing oxidative stress. Coumarins and tannins have also been shown to interfere with fungal enzyme systems [23]. These findings are consistent with other studies, such as Behiry et al. 2022, which highlighted the presence of phenolic-rich fractions in taro and turmeric extracts correlating with strong antifungal and anti-mycotoxin activity [24].

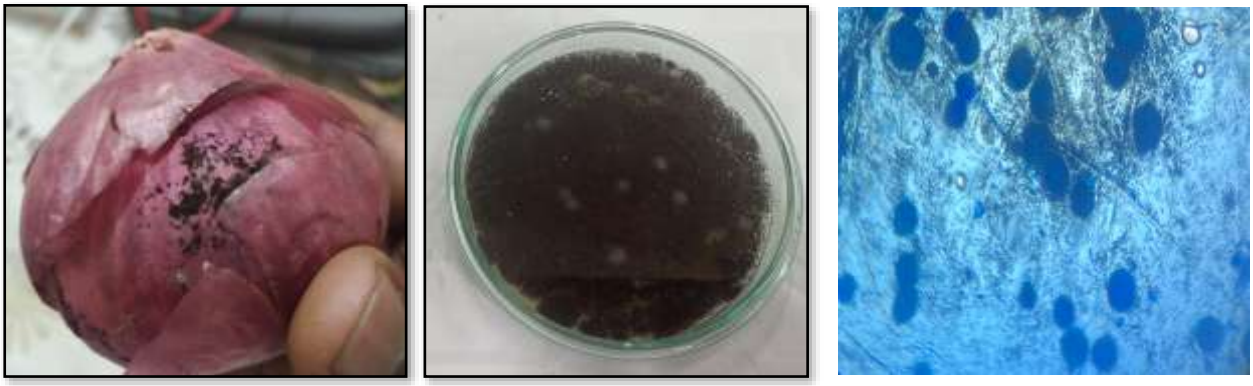

Figure 1: Infected Onion Bulb Showing Black Rot Caused by *Aspergillus* spp. 2] Pure Culture of *Aspergillus* spp. Grown on Potato Dextrose Agar [PDA] Plate 3] Microscopic View of Aspergillus spp. Stained with Lactophenol Cotton Blue Showing Characteristic Conidiophores

Sample Code	Dry Weight [mg]	Percentage Yield [%]
Ws-Ace	81	2.025
Ws-MeOH	86	2.15
Ws-Aq	101	2.525
Pb-Ace	107	2.675
Pb-MeOH	110	2.75
Pb-Aq	135	3.375
Cv-Ace	98	2.45
Cv-MeOH	124	3.10
Cv-Aq	129	3.225
Li-Ace	117	2.925
Li-MeOH	110	2.75
Li-Aq	115	2.875

Table 1: Percentage yield of Plant extracts in various solvents

Plant Extract: ***Ws*** = *Withania somnifera*, ***Pb*** = *Piper betle*, ***Cv*** = *Cinnamomum verum*, ***Li*** = *Lawsonia inermis*.
Solvent Abbreviations: ***Ace*** = Acetone, ***MeOH*** = Methanol, ***Aq*** = Aqueous [Distilled Water]

Test→ Sample	Alkaloids	Flavonoids	Phenolic	Tannins	Coumarins
Ws-Ace	–	+	–	–	+
Ws-MeOH	+	+	–	–	+
Ws-Aq	+	+	–	–	+
Pb-Ace	–	+	+	+	+
Pb-MeOH	+	–	+	+	+
Pb-Aq	+	-	+	+	+
Cv-Ace	–	+	–	–	+
Cv-MeOH	+	+	–	–	+
Cv-Aq	+	-	–	–	+
Li-Ace	–	+	+	+	+
Li-MeOH	+	+	+	+	+
Li-Aq	+	+	+	+	+

Table 2: Preliminary Phytochemical Screening of Plant Extracts. [+] indicates presence; [–] indicates absence of the respective phytochemical.

Sample	Zone of Inhibition [mm]
Ws-Ace	–
Ws-MeOH	–
Ws-Aq	–
Pb-Ace	1.4 cm [14 mm]
Pb-MeOH	1.2 cm [12 mm]
Pb-Aq	–
Cv-Ace	12mm ± 0.1
Cv-MeOH	–
Cv-Aq	–
Li-Ace	–
Li-MeOH	–
Li-Aq	–
NC -Ace	–
NC-MeOH	–
NC-Aq	–
PC- Fluconazole	

Table 3: Zone of Inhibition [mm] of Selected Plant Extracts and Fluconazole Against *Aspergillus niger.*

The antifungal activity of the plant extracts was evaluated against *Aspergillus niger* using the agar well diffusion method at a standardized concentration of 0.25 mg/mL. Among all tested extracts, the acetone extract of *Piper betle* [Pb-Ace] produced the highest inhibition zone of 14 mm, followed by the methanol extract [Pb-MeOH] at 12 mm, and the acetone extract of *Cinnamomum verum* [Cv-Ace] at 12 mm ± 0.1. No inhibition was observed for aqueous extracts or for the remaining plant samples at this concentration. As seen in Fig. 3.

Despite the known efficacy of fluconazole, its incomplete inhibition of *A. niger* at 0.25 mg/mL underlines the need for alternative or adjunct antifungal therapies. These results strongly correlate with the phytochemical content and extraction solvent. Organic solvents like acetone and methanol effectively extracted phenolic and flavonoid compounds [25,26]. This is also evident in both our phytochemical screening and antifungal bioassays. Similar observations were made in earlier research by Surapuram et al.

[2014], where plant extracts in acetone and methanol demonstrated superior antifungal activity with MIC values below 100 µg/mL against *A. niger*.

Furthermore, a study by Zenat et al. [2024] reported strong inhibition [up to 97.6%] by methanolic plant extracts at 15% concentrations against *Aspergillus* spp.,[27] which supports our finding that methanolic extracts of *P. betle* can be potent even at significantly lower concentrations [0.25 mg/mL]. The lack of antifungal activity in aqueous extracts, despite reasonable yield, further validates that water is inefficient in extracting key antifungal phytochemicals such as alkaloids, saponins, or terpenoids. This is consistent with findings from previous studies involving *Allium* species, where alcoholic garlic extracts showed superior activity over aqueous forms.

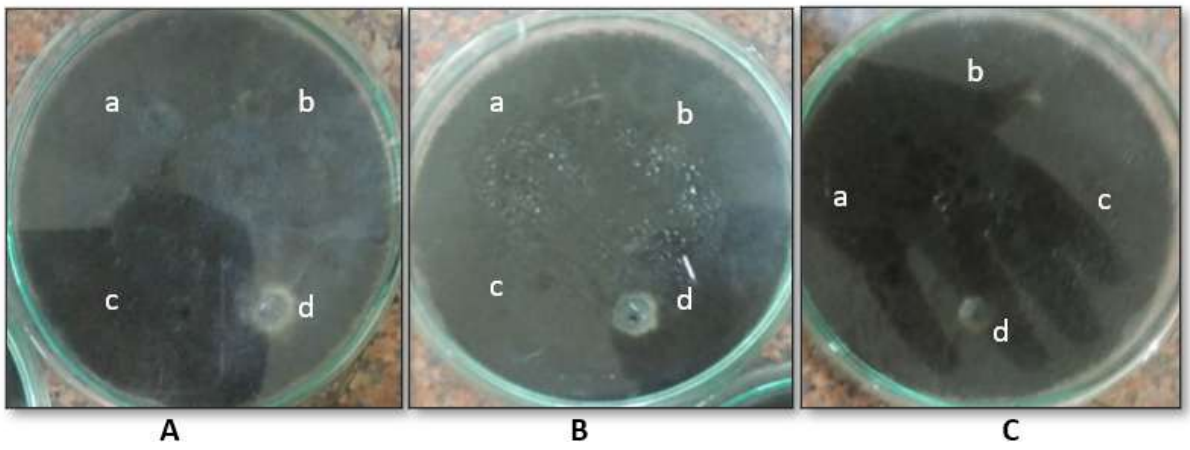

Fig 3: Visualization of inhibition zones for plant extracts against *A. niger*: [A] *P. betle* acetone, [B] *P. betle methanol*, [C] *C. verum* acetone. [a – Solvent control, b – Positive control [Fluconazole], c – Distilled water control, d – Plant extract in respective solvent].

CONCLUSIONS

Overall, the results highlight the significant influence of solvent polarity on both phytochemical extraction and biological activity, affirming that acetone and methanol are more suitable solvents for isolating antifungal agents from medicinal plants. The acetone extract of *Piper betel* showed the strongest antifungal activity among all tested samples, followed by its methanolic counterpart and the acetone extract of *Cinnamomum verum*, indicating a clear correlation between extract solvent, phytochemical richness, and antifungal efficacy.

Although the standard antifungal drug fluconazole produced a larger inhibition zone, it did not completely suppress fungal growth, suggesting emerging tolerance of *Aspergillus niger* at lower drug concentrations. In contrast, some of the plant extracts, particularly *P. betle*, demonstrated noteworthy inhibition at a similar concentration [0.25 mg/mL], underlining their potential as natural, cost-effective antifungal alternatives.

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