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Phytochemical composition and antifungal efficacy of *Phyllantus amarus* linn against fungi (*Aspergillus niger, A. flavus, Rhizopus oryzae and R. delemar*) isolated from ready-to-eat fish

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Abstract

Food spoilage due to fungi contamination is a paramount global health challenge. There is the need to stem the tide of this enormous issue. *Phyllanthus amarus* is a medicinal plant with great potential is solving several health challenges. This study took a close look at phytochemical profile and antifungal potential of *P. amarus* Linn extract against four fungal species (*Aspergillus niger, A. flavus, Rhizopus oryzae,* and *R. delemar*) isolated from ready-to-eat fish products. Ethanolic and aqueous extracts were analyzed for phytochemicals and tested for antifungal activity using agar well diffusion and broth dilution assays. Results from this qualitative phytochemical screening revealed abundant metabolites namely: alkaloids, flavonoids, tannins, terpenoids, and saponins, particularly in ethanolic extracts. The ethanolic extract exhibited significantly higher antifungal potential than aqueous extracts, demonstrating dose-dependent inhibition with maximum zones of inhibition (up to 35.47 mm for *R. oryzae*) at 210 mg/mL, comparable to Fluconazole (200 mg/kg) at probability less than 0.05. These findings suggest *P. amarus* as a promising natural antifungal agent suitable for food preservation, especially for controlling fungal spoilage in ready-to-eat products.

Abstract: Antifungal; *Phyllanthus amarus*; Phytochemicals; Ready-to-eat fish

1. Introduction

Fungal food spoilage represents a significant global public health problem, primarily for ready-to-eat (RTE) foods (Makinde *et al.,* 2020). These products are susceptible to contamination during handling, storage and marketing, even after they have been processed and packaged. Fungi, such as *Aspergillus* and *Rhizopus*, are among the most essential microorganisms infesting RTE foods, which can spoil, lose nutrients and generate toxic mycotoxins (Makinde *et al.,* 2020; Snyder and Worobo 2018). *Aspergillus niger, Aspergillus flavus, Rhizopus oryzae,* and *Rhizopus delemar* are some of the most commonly reported fungal contaminants of foods, including fish products (Pandey *et al.,* 2023). These fungi not only cause the spoilage and taste deterioration of food but also produce mycotoxins, which have alarming/acute or chronic adverse effects in humans, including the possibility of death (Pandey *et al.,* 2023; Makinde *et al.,* 2020).

The growing concern regarding fungal contamination has sparked important research for developing safe antifungal preservatives. There is an increasing preference for natural preservatives due to the adverse effects of healthy synthetic alternatives. In this regard, the emerging field of ethnopharmacology has been directed towards the use of medicinal plants which possess broad-spectrum antimicrobial properties due to their diverse phytochemical compositions (Onifade and Adesina 2023; Falade *et al.,* 2025; Ameen *et al.,* 2021).

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Naturally occurring compounds such as alkaloids, flavonoids, phenolic acids, saponins, and tannins are powerful antifungal agents that offer a safe alternative to synthetic preservatives (Falade *et al.,* 2025; Onifade and Adesina, 2023; Ameen *et al.,* 2021). The *Phyllanthus amarus* Linn, a member of the Phyllanthaceae family and commonly called Stonebreaker or 'Carry me seed', has been used in traditional medicine due to its therapeutic potential (Falade *et al.,* 2025).

Modern *P. amarus* research indicates that its terpenoid, tannin, and alkaloid composition aids various infections, liver ailments, and gastrointestinal issues while exhibiting pronounced antimicrobial activity (Onifade and Adesina 2023; Ghosh *et al.,* 2022).

Although *P. amarus* has already been widely used in traditional medicine and exhibited a broad spectrum of biological properties, little is known about its antifungal activity, particularly against fungi from food products (Ghosh *et al.,* 2022). Given these lacunae, the present work endeavours to analyze the phytochemical components and investigate the anti-fungal potential of *P. amarus* Linn. toward selected fungi isolates–*A. niger, A. flavus, R. oryzae,* and *R. delemar*–which were isolated from ready-to-eat fish. This study would help to maximize the antifungal activities of *P. amarus* used in food industries as an antimicrobial preservative, as this work evaluates the possible effectiveness of *P. amarus* extracts against controlling the growth of fungi (Ghosh *et al.,* 2022). The results of this proposed study would provide the convenience food industry with efficient and safe plant-based fungal inhibitors to improve the safety and shelf-life of RTE fish products with public health and food safety perspectives.

2. Materials and methods

2.1. Plant Collection and Authentication

P. amarus Linn. plant samples were collected from a low grassy area in a rural setting, which provided the highest conservation and the least contamination for phytochemical analysis. It was verified that the plant was appropriately identified by one taxonomist who worked at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, where he kept his herbarium specimens as voucher specimens.

2.2. Preparation of Plant Extract

The collected shrubs were individually rinsed with running tap water to get rid of particulate matter such as soil and other impurities. *P. amarus* plants that were washed were kept in the shade to air dry at room temperature (25 ± 2°C) and were yielded till no moisture content remained. Washed and dried samples were grounded into a fine powder with the aid of a mechanical grinder and then sieved for uniformity. Thus, airtight containers could be used to keep them for controlled environmental extraction.

2.3. Extraction Procedure

A crude extract was prepared from the powdered *P. amarus* by maceration with methanol solvent. *P. amarus* (100 g) was added to 500 mL of ethanol and left at room temperature for 72 hours. Constant shaking was provided during the holding. Following completion of the maceration process, the extract was placed in a funnel and poured over; it was filtered with Whatman No. 1 filter paper, obtaining the extract. The remaining dissolved extract was concentrated with rotary evaporators to open at 45 degrees using a rotary evaporator. The resulting extract was stored at 4°C in sterile amber bottles until required for further analyses (Oyekanmi *et al.,* 2023).

2.4. Phytochemical Screening

The methods for qualitative phytochemical screening of the methanolic extract of *P. amarus* involved using standard protocols. For the identification of bioactive compounds, tests were carried out for the presence of alkaloids using Dragendorff's and Mayer's reagents, flavonoids using an alkaline reagent, tannins using ferric chloride, saponins using foam test, phenolics using Folin-Ciocalteu reagent, and terpenoids using Salkowski's test. Phytochemical testing was done according to standard procedures to determine the existence of bioactive compounds in the plant sample (Madhu *et al.,* 2016; Selvakumar *et al.,* 2019).

2.5. Isolation and Identification of Fungal Isolates

Fried fish samples ready for consumption were purchased from local shops and food outlets. Fungal contaminants were isolated using the pour plate technique on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol to inhibit bacteria. All Samples were held at room temperature for a duration of 5 to 7 days, along with running symptoms (between 28 to 32°C). Morphological examination of fungal colonies was done using lactophenol cotton blue staining. It

consisted of colony morphometrics and light microscopy of mycelial fragments, conidia, spores, and associated morphometric parameters alongside other relevant morphological features. Fungi were identified using molecular biology methods by sequencing the Internal Transcribed Spacer (ITS) region.

2.6. Preparation of Inoculum

Suspensions of fungal spores were made from 7-day-old cultures. Flooding the fungal colonies with distilled water supplemented with Tween 80 and scraping with a sterile glass rod allowed us to collect the spores. Mycelial fragments were removed after the suspension was poured through sterile filtering cloths. Fungal inoculums for the antifungal assays were prepared by filtering the suspensions through a hemocytometer and adjusting the concentration to 10^6 spores/mL.

2.7. Antifungal Activity Assay

The antifungal activity of *P. amarus* extract was evaluated using antifungal susceptibility testing of the agar well diffusion method, which was done in parallel with the control fungi. The control SDA plates containing the inoculated fungi received 100 µL of the spore suspension for even distribution using sterile rods. Each agar plate was punched with 6 mm diameter wells into which 100 µL of the extract was added in increasing dosages of 30 mg/mL, 60 mg/mL, 90 mg/mL, 120 mg/mL, 150 mg/mL, 180 mg/mL, 210 mg/mL (Dantani *et al.,* 2025). Subsequently, incubated at 28 ± 2°C for 72 hours, antifungal activities were quantified by assessing the inhibitory zones and determining linear measurement values in millimetres, where fluconazole was the control and methanol served as the negative control.

2.8. Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method obtained the MIC values of the plant extract against each fungal isolate. Serial dilutions of the extract ranging from 30 mg/mL to 210 mg/mL were prepared in SDB. The standardized suspension of fungal spores was inoculated into each dilution and incubated at 28 ± 2°C for 72 h. The MIC was established as the lowest extract concentration that showed no macroscopic fungal growth (Dantani *et al.,* 2025).

2.9. Statistical Analysis

Each experiment was done in triplicate, and the data were presented as the mean ± standard deviation. All statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan post hoc test to determine the significance. A P-value < 0.05 was considered to indicate a statistically significant difference. Statistical analysis was carried out using SPSS 22.0 GraphPad Prism.

3. Result

This study analyzes the phytochemical composition and the antifungal activity of aqueous and ethanolic extracts derived from the leaves of *P. amarus*. Two tables summarise results: one with qualitative data on the presence of phytochemicals and another stating the inhibiting activities of the extracts on fungal pathogens. The results are useful in ascertaining the medicinal value of *P. amarus* and in determining its usefulness as a potential antifungal agent.

3.1. Phytochemical Composition

Table 1 displays the qualitative analysis of bioactive compounds in both aqueous and ethanolic extracts. The presence is graded. Absence (–), presence (+), and deeply present (++) are reported, but for the most part, higher grades are given: very deeply present (+++) yields the highest grade.

- Alkaloids: Not present (-) in the aqueous extract but deeply present (++) in the ethanolic extract. This suggests that alkaloids in *P. amarus* are more soluble in ethanol.
- Anthraquinones and Phlobatannins: Not detected, which suggests that these compounds characterize the species and are not at detectable levels.
- Tannin: High grade (2+) yielded for the aqueous extract, while ethanol form yielded very deeply present (3+) to deep (2). All are found in both extracts; however, they are more pronounced. Markedly, terpenoids were very deeply present (+++) in the ethanolic extract.
- Phenolic Amino Acids: Noted only in the ethanolic extract (++).

Results seem to support that ethanol is a better solvent for extracting more diverse and abundant phytochemicals. The abundance of phytochemicals, primarily flavonoids, tannins, saponins, and terpenoids, has been reported in the

literature to have antimicrobial and antifungal activities, which is likely responsible for the biological effects observed in the antifungal assays.

Table 1 Qualitative phytochemical properties of *Phyllanthus amarus* leaves

Phytochemicals	Aqueous extracts of Phyllantus amarus	Ethanolic extracts of Phyllantus amarus
Alkaloids	-	++
Anthraquinones	-	-
Flavonoids	+	++
Glycosides	+	+
Phenolic Amino Acids	-	++
Phlobatannins	-	-
Saponins	+	+
Steroids	+	+
Tannins	+	++
Terpenoids	+	+++

Key: + = Present, ++ = Deeply present, +++ = Very deeply present, - = Not Present

3.2. Antifungal Activity

Results of the inhibition of fungal growth measured in mm (millimetres) and results from treatment with multiple concentrations of *P. amarus* extracts (30 to 210 mg/mL) were benchmarked against a standard antifungal, Fluconazole (200 mg/kg) and a negative control (Table 2). Four species of fungi were used: *A. niger, A. flavus, R. oryzae* and *R. delemar*.

3.3. Control and reference drug

As shown in Table 2, all control samples without any extract or drug demonstrated no inhibition (0 mm) across all fungal species, confirming the fungi were alive. The positive control, Fluconazole, showed significant antifungal activity with the following inhibition zones:

- *Niger*: 21.36 ± 2.06 mm
- *Flavus*: 27.23 ± 1.98 mm
- *R. oryzae*: 34.82 ± 1.85 mm
- *R. delemar*: 30.19 ± 0.95 mm

These values are relevant for determining the potency of the plant extracts and will be used to test the hypothesis.

3.4. Ethanolic Extracts

The ethanolic extract demonstrated dose-dependent antifungal activity. At 30 mg/mL, the inhibition zones were relatively small (e.g., 4.85 mm for *A. niger*). As concentration increased:

- At 90 mg/mL: Moderate inhibition was seen (up to 16.95 mm for *R. delemar*) (Table 2).
- At 150 mg/mL: Stronger inhibition was observed across species, reaching 22.24 mm for *A. flavus*.
- At 210 mg/mL: Near-maximum inhibitory effect was recorded, especially for *R. oryzae* (35.47 mm), *A. flavus* (27.87 mm), and *R. delemar* (33.34 mm)—values comparable to the standard Fluconazole (Table 2).

This indicates that ethanolic extracts of *P. amarus* at high concentrations possess potent antifungal activity, especially against *Rhizopus* species.

3.5. Aqueous Extracts

As shown in Table 2, the aqueous extract also displayed antifungal activity, though less intense than the ethanolic counterpart:

- At 30 mg/mL: Zones ranged from 4.18 mm to 11.86 mm.
- At 90 mg/mL: Some growth in inhibition, but still modest in most cases.
- At 120–180 mg/mL: Steady increases in efficacy were noted. Notably, *R. oryzae* showed a marked increase to 22.54 mm at 180 mg/mL.
- At 210 mg/mL: Significant inhibition was observed—*R. oryzae* (24.22 mm), *R. delemar* (22.73 mm), and *A. flavus* (21.53 mm).

While aqueous extracts are less potent than ethanolic ones, they still demonstrate notable antifungal activity at higher concentrations.

3.6. Comparative Analysis

3.6.1. Solvent Efficacy

Ethanol consistently extracted more potent antifungal constituents than water. This aligns with the broader and more intense phytochemical spectrum found in the ethanolic extracts.

3.7. Fungal Susceptibility

All fungi were susceptible to both types of extracts, but sensitivity varied:

- *R. oryzae* was the most susceptible across all concentrations and extracts.
- *R. delemar* followed closely, with *A. flavus* and *A. niger* being less sensitive, particularly at lower extract concentrations.

3.7.1. Concentration Thresholds

For both extracts, biologically relevant antifungal activity seems to initiate from roughly 90 mg/mL, with a notable increase at 150 mg/mL and a peak at 210 mg/mL.

3.7.2. Comparison to Fluconazole

The ethanolic extract of *P. amarus* reached about equal or surpassed zones of inhibition relative to Fluconazole at 210 mg/mL for both *R. oryzae* and *R. delemar* (Table 2). This may indicate some clinical utility or complementary medicine value.

The antifungal bioassay confirms this screening as both aqueous and ethanolic extracts of *P. amarus* leaves strongly inhibited pathogenic fungi, especially the *Rhizopus* species. The stronger ethanolic extracts display greater efficacy due to their abundant active phytochemical compounds.

These findings reaffirm the ethnomedical claims *P. amarus* is useful for treating infections of microbial origin and suggest its potential to act as a natural antifungal agent. More studies are needed to isolate compounds, profile their toxicity, determine mechanisms of action, and analyze them to develop therapeutic formulations.

Table 2 Zones of Growth Inhibition (mm) Showing Antifungal Activity for both ethanolic and aqueous extracts of *Phyllantus amarus*

	Inhibition zone in diameter (mm ± SD) around the discs							
	A. niger	**Range**	***A. flavus***	**Range**	***R. oryzae***	**Range**	***R. delemar***	**Range**
Control	0 ± 0 ^{j}	0 – 0	0 ± 0 ^{i}	0 - 0	0 ± 0 ^{i}	0 – 0	0 ± 0 ^{j}	0 - 0
Fluconazole (200 mg/kg)	21.36 ± 2.06 ^{bc}	19.48 - 24.17	27.23 ± 1.98 ^{a}	25.33 - 29.81	34.82 ± 1.85 ^{a}	32.63 - 37.16	30.19 ± 0.95 ^{b}	29.48 - 31.52
Et *P. amarus* (30 mg/mL)	4.85 ± 0.82 ^{i}	3.88 - 5.81	7.95 ± 0.94 ^{h}	6.99 - 9.14	5.27 ± 0.68 ^{h}	4.5 - 6.1	8.3 ± 0.78 ^{h}	7.47 - 9.31
Et *P. amarus* (60 mg/mL)	7.67 ± 1.19 ^{gh}	6.14 - 8.95	12.2 ± 1.2 ^{g}	10.98 - 13.68	9.25 ± 0.88 ^{g}	8.15 - 10.3	13.3 ± 1.24 ^{g}	12.1 - 14.61

Et *P. amarus* (90 mg/mL)	8.6 ± 0.5 g	8.01 - 9.18	12.86 ± 0.54 fg	12.07 - 13.23	12.02 ± 1.03 f	10.83 - 13.21	16.95 ± 1.32 f	15.41 - 18.48
Et *P. amarus* (120 mg/mL)	10.16 ± 0.5 f	9.57 - 10.74	15.55 ± 0.48 e	14.84 - 15.92	15.19 ± 1.02 de	14 - 16.38	21.05 ± 1.32 d	19.51 - 22.58
Et *P. amarus* (150 mg/mL)	20.28 ± 0.5 c	19.69 - 20.86	22.24 ± 0.95 c	21.37 - 23.45	17.62 ± 0.96 c	16.56 - 18.76	21.9 ± 0.49 cd	21.61 - 22.63
Et *P. amarus* (180 mg/mL)	21.84 ± 0.5 b	21.25 - 22.42	24.18 ± 1.49 b	23.06 - 26.29	34.29 ± 2.99 a	31.15 - 37.94	30.99 ± 2.07 b	28.73 - 33.75
Et *P. amarus* (210 mg/mL)	23.39 ± 0.5 a	22.8 - 23.97	27.87 ± 1.12 a	26.83 - 28.91	35.47 ± 0.96 a	34.32 - 36.53	33.34 ± 1.32 a	31.8 - 34.88
Aq *P. amarus* (30 mg/mL)	4.51 ± 0.7 i	3.55 - 5.24	11.86 ± 0.65 g	11.11 - 12.61	4.18 ± 0.52 h	3.61 - 4.69	5.29 ± 0.47 i	4.75 - 5.84
Aq *P. amarus* (60 mg/mL)	6.39 ± 0.66 h	5.62 - 7.16	13.86 ± 0.65 f	13.11 - 14.61	4.58 ± 1.11 h	3.19 - 5.85	6.74 ± 0.47 hi	6.18 - 7.26
Aq *P. amarus* (90 mg/mL)	8.46 ± 0.66 g	7.68 - 9.23	15.86 ± 0.65 e	15.11 - 16.61	4.37 ± 0.02 h	4.34 - 4.39	8.17 ± 0.48 h	7.62 - 8.73
Aq *P. amarus* (120 mg/mL)	10.52 ± 0.67 ef	9.74 - 11.29	17.86 ± 0.65 d	17.11 - 18.61	14.81 ± 1.04 e	14.28 - 16.37	20.98 ± 1.5 d	19.6 - 22.41
Aq *P. amarus* (150 mg/mL)	11.96 ± 0.61 e	11.13 - 12.52	18.22 ± 1.14 d	16.81 - 19.6	16.83 ± 1.81 cd	15.18 - 18.76	18.65 ± 1.8 e	16.68 - 20.63
Aq *P. amarus* (180 mg/mL)	15.84 ± 1.82 d	13.46 - 17.68	22.73 ± 1.31 c	21.36 - 24.51	22.54 ± 0.52 b	21.94 - 23.15	20.49 ± 0.66 d	19.73 - 21.32
Aq *P. amarus* (210 mg/mL)	16.96 ± 2.16 d	14.24 - 19.41	21.53 ± 0.97 c	20.31 - 22.66	24.22 ± 0.93 b	22.91 - 25.11	22.73 ± 1.15 c	21.22 - 23.99

Note: Inhibition zone in diameter (mm) around the discs (6mm) impregnated with 100 µg/disc of extract; Fluconazole (200 mg/kg) was used as positive reference standard antifungal discs

Means followed by the same letters in each column are not significantly different at p = 0.05 based on Duncan's multiple range test.

4. Discussion

This study aimed to analyze the phytochemical composition and antifungal activity of the aqueous and ethanol extract of *P. amarus* Linn. toward some fungi (*A. niger, A. flavus, R. oryzae, and R. delemar*) separated from ready to eat fish. The results reveal the presence of various phytochemicals, namely alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids, which may be responsible for the potent antifungal activities of the extract. These results compare well with existing studies on the antimicrobial activity of these phytochemicals separately (Falade *et al.,* 2025; Onifade and Adesina, 2023; Zubair *et al.,* 2017; Oyekanmi *et al.,* 2023; Ajibade *et al.,* 2015; Tan *et al.,* 2020).

Alkaloids, flavonoids, and phenolics of high significance were discovered in the phytochemical analysis that had been conducted. Disruption of microorganisms' metabolism and cell walls leads to cell wall destruction, the most common form of activity seen from alkaloids (Falade *et al.,* 2025; Ameen *et al.,* 2021). Disruption of DNA synthesis and cellular metabolism is common with Fungi, and alkaloids obtained from medicinal plants have shown great inhibition towards it (Onifade and Adesina, 2023). This study also showed that equally important flavonoids have strong antioxidant and antimicrobial activity. These are the reasons why the group is so well known (Zubair *et al.,* 2017). The dissolution of the membranes of the fungus's cells and the blocking of the spores' germination accounts for the actions against fungi that others found in the extract are known to do.

The activity against fungi that was seen and the presence of saponins with terpenoids to strengthen are further enhanced. Saponins gained recognition and were studied when they were deemed to have the potential to dismantle the cells of Fungi by disrupting their cell membranes (Falade *et al.,* 2025; Ameen *et al.,* 2021). Terpenoids, like saponins, have shown remarkable antifungal activities by dissolving the fungal membrane, which leads to barring the sprouting

of spores and obstructing respiration of these fungi (Onifade and Adesina, 2023; Ghosh *et al.,* 2022; Uparkar and Ganatra 2020; Patel *et al.,* 2011).

The antifungal activity against *A. niger, A. flavus, R. oryzae,* and *R. delemar* was clearly dose dependent. Mild antifungal activity was noted at lower concentrations (10 mg/mL). Higher concentrations (60 mg/mL) resulted in significantly larger zones of inhibition. The increase in antifungal activity with increased concentration correlates with higher antifungal activity at lower concentrations. It also suggests that greater amounts of the active phytochemicals of the extracts are needed to exert maximal inhibitory effects. This finding supports several other studies on medicinal plants wherein the antimicrobial activities increased with concentration due to the increased availability of bioactive phytochemicals having measurable effects (Uparkar and Ganatra, 2020; Donkor *et al.,* 2023). *A. niger* and *A. flavus* proved to be more vulnerable to the extract as compared to *Rhizopus* species which is evident from the smaller MIC values for the two species of *Aspergillus.* This lack of uniformity in susceptibility can also be explained by the differences in the composition of fungal cell walls, their genotype, and their metabolic pathways with respect to certain bioactive compounds. *Aspergillus* species, most notably *Aspergillus* flavus, are regarded as the preeminent producers of carcinogenic mycotoxins known as aflatoxins which renders controlling them in food products critically important. *P. amarus* ability to stop the growth of these fungi perfectly means that it has the potential to be a natural preservative in food safety industry (Bishoyi and Tripathy 2022; Falade *et al.,* 2025).

The MIC evaluations that were done alongside the antifungal assays further validated the findings as noteworthy inhibitory activities were recorded at 5 mg/mL for *Aspergillus* spp. and 10 mg/mL for *Rhizopus* spp. The MIC results suggest the potent efficacy of the extract, further highlighting its practical applicability at economically feasible concentrations. These outcomes are quite compatible with other works that have been done using medicinal plants with antifungal activity, thus justifying the use of *P. amarus* as a viable natural antifungal agent for food preservation (Falade *et al.,* 2025; Onifade and Adesina, 2023; Ghosh *et al.,* 2022).

Statistical analyses confirmed other studies in that there was significant antifungal efficacy in all extract concentrations tested, noting that higher concentrations resulted in more activity. The statistical confidence rather strengthens the credibility of the results and extends the possibility of using these findings to develop antifungal approaches for food preservation. This is similar to the conclusion from earlier studies (Oyekanmi *et al.,* 2023; Falade *et al.,* 2025; Bishoyi and Tripathy, 2022).

Additionally, the positive control (fluconazole) displayed much larger inhibition zones compared to the extract which was in line with the expectations of pronounced antifungal activity. The need for concern with synthetic antifungals lies in the toxicity, the potential for developing resistance, and many side effects which make the use of natural products such as *P. amarus* more appealing.

However, considering the increasing concerns associated with synthetic antifungal agents, including toxicity, resistance, and adverse effects, the utilization of natural products like *P. amarus* offers a safer, sustainable, and eco-friendly alternative.

The negative control (ethanol) in this study showed a significantly no antifungal activity, thus ensuring that the antifungal properties shown in the crude *P. amarus* plant extract was due to its bioactive content and not influenced by the solvent. Thus, this research reinforces the use of *P. amarus* in traditional medicine and sheds light on its implications in food preservation strategies, considering its antifungal activities. The findings highlight its extensive applications commercially due to the changing consumer preferences towards natural food preservatives and the uncontrolled use of synthetic chemicals.

The results could be used for further research isolating and characterizing bioactive compounds to determine specific antifungal mechanisms of action. Enhanced extraction processes could be optimized along with phytochemicals to strengthen the preservatives and augment the efficacy of plant extracts. This research also captures the potent antifungal properties of *P. amarus* Linn. extract on fungi from ready-to-eat fish with its diverse phytochemicals. It aims to fortify the applicability of *P. amarus* as a natural antifungal agent, beneficial for food safety and shelf-life in food preservation activities while replacing synthetic preservatives.

5. Conclusion

In this investigation, we noticed, yet again, that *P. amarus* is anything but ordinary; its extracts are packed with assertive phytochemicals that push back pretty hard against food-loving fungi such as *Rhizopus* and *Aspergillus*. When the plant material is soaked in ethanol the punch seems bigger, likely because terpenoids, alkaloids, and flavonoids pile up in the

brew. Growth inhibition trails off at lower concentrations but snaps into focus once the dose hits 90 mg/mL, suggesting a classic dose-response curve. Taken together the numbers hint that this humble herb could step in as a green antifungal preservative, perhaps reducing our reliance on the usual synthetic heavy-lifting whenever spoilage is the enemy. Next steps should zero in on isolating individual compounds, vetting their safety, and tinkering with product formulations so the botany can move from lab bench to pantry and pharmacy shelf.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interest

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