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COMPARATIVE ANTIFUNGAL EFFICACY OF TWO SELECTED INDIGENOUS PLANT EXTRACTS AGAINST POSTHARVEST COCOYAM PATHOGENS IN MAJOR MARKETS IN ANAMBRA STATE.

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Abstract

This research evaluated the comparative antifungal efficacy of selected indigenous plant extracts against postharvest fungal pathogens associated with cocoyam tuber spoilage in major markets in Anambra State. Plants selected for this research includes *Vernonia amygdalina* and *Hyptis suaveolens* leaves. The aim of this study is to establish the usage of indigenous plant extracts to control pathogenic fungi distroying farm produce . Cocoyam tuber were collected from different markets in Anambra State. Standard Association of Official Analytical Chemists methods (AOAC) were employed for fungal isolation, while *in vitro* antifungal assays were conducted using graded extract concentrations. Five spoilage fungi were consistently isolated and identified: *Aspergillus flavus*, *Aspergillus Niger*, *Fusarium solani*, *Rhizopus stolonifer*, and *Penicillium expansum*. Tabulated results of Phytochemical screening revealed the presence of some bioactive compound in the so tested plant extracts in varying concentrations. *In vitro* assays demonstrated significant antifungal effects ($p < 0.05$), with inhibition zones increasing with extract concentration (100% > 50% > 25%). *V. amygdalina* exhibited higher inhibitory activity against most isolates than that of *H. suaveolens* . The results confirm that ethanol extracts of these plants possess potent antifungal properties and can serve as eco-friendly alternatives or supplements to synthetic fungicides.

Keywords: Cocoyam, indigenous plant, phytochemical, bioactive compounds, *Hyptis suaveolens, Fusarium solani*

INTRODUCTION

Cocoyam (*Colocasia esculenta L. Schott*) is a herbaceous perennial monocotyledon belonging to the family Araceae and the genus *Colocasia*(Onwueme, 2019). It is believed to have originated from South and Central Asia and later dispersed across tropical Asia and Oceania more than 2,000 years ago, where it became a culturally and economically significant food crop (Anukwuorji *et al.*, 2012). Today, cocoyam is cultivated in many parts of Africa, Asia, and the Pacific Islands and continues to play an important role in food security due to its high carbohydrate yield and adaptability to diverse agro-ecological conditions (Eze and Okorji, 2020).

In Nigeria, two major cocoyam species are cultivated: *Colocasia esculenta* (locally called ede-uli) and *Xanthosoma sagittifolium* (ede-oku). *C. esculenta* predominates in the southeastern states, particularly in Anambra, where it is a dietary staple and is often used as a thickener in soups, while *X. sagittifolium* is less popular but consumed boiled or pounded (Chayty *et al*., 2017). Cocoyam provides a substantial proportion of dietary carbohydrates in rural households, but its production faces serious challenges, particularly postharvest deterioration.

Post-harvest loss of root and tuber crops has been a very serious problem to farmers as more than 40% of their harvest may be lost because of decay (Olurinola *et al*., 2020). Studies have shown that fungal rot is the greatest cause of root and tuber loss in storage (IITA, 2015).

The principal species of microorganisms associated with cocoyam rot in Nigeria include *Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae, Sclerotia rolfsii*, *Fusarium solani and Erwinia carotovora*, these fungi werereported to be pathogenic to four cultivars of *Colocasia esculenta*, causing rot of cocoyam in several parts of southern Nigeria (Eze and

Nwanguma, 2013). According to reports, these fungal pathogens are also the main causes behind storage rots in other root and tuber crops including yam and cassava. (Okigbo *et al*.,2010; Eze and Ameh, 2011) Recent studies in Anambra State confirm the presence of these fungi in cocoyam sold in major markets, with *Rhizopus stolonifer* and *Phytophthora parasitica* also reported as causative agents of spoilage (Ejimofor and Okigbo, 2023).These fungi not only deteriorate tuber texture and taste but also produce mycotoxins that reduce nutritional quality and pose public health risks.

Conventionally, chemical fungicides have been employed to mitigate postharvest fungal infections. While effective, their use is increasingly discouraged due to high cost, residue accumulation, environmental toxicity, and the emergence of resistant fungal strains (Okigbo *et al*., 2015). This has created a strong demand for eco-friendly alternatives. Plant-based extracts, rich in bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolics, and essential oils, have shown promising antifungal activity against several plant pathogens (Timothy *et al.*, 2018; Ejimofor and Okigbo. 2023) They are locally available, affordable, biodegradable, and less hazardous compared to synthetic fungicides, making them attractive options for sustainable crop protection.

Unlike synthetic chemicals, these compounds are biodegradable, eco-friendly, and often less toxic to humans and animals. Furthermore, many of these plants are locally available in Nigeria, making them accessible and affordable for smallholder farmers. According to Iwuagwu *et al*. (2018) fungicidal plant are highly effective at preventing fungal growth both in vitro an in vivo. Several indigenous plants have been evaluated in Nigeria for their fungitoxic potential relevant to the control of postharvest pathogens of root and tuber crops. This creates a technological gap in safe and affordable postharvest management strategies for smallholder farmers. The exploration of

plant-derived biofungicides offers a promising alternative, as medicinal plants such as *Vernonia amygdalina* (bitter leaf) and *Hyptis suaveolens*(bush mint) have been reported to possess potent antifungal properties (Ugbogu *et al.*, 2022; Okigbo and Emoghene, 2024).

Materials and Method

Collection of Samples

Samples of spoilt cocoyam (*Colocasia esculenta*) tubers were collected from twelve (12) distinct markets across Anambra State. The samples were carefully collected using sterile sample bags to minimize contamination. Each sample was clearly labeled with the name of the market and date of collection before being transported to the laboratory for analysis.

The plant materials used for the study were *Vernonia amygdalina and Hyptis suaveolens* leaves. These were collected from farms and natural habitats in Anambra State using clean collecting bags to ensure that the products were free of other pollutants.

The specimens were collected and taken to the Department of Biological Sciences Chukwuemeka Odumegwu Ojukwu University, Uli for identification and verification by a botanist, Prof. C G. Ukpaka.

Culture Media

Two commercially available media were employed for the isolation of fungi. PDA was used as a general-purpose medium suitable for the growth of a wide range of fungi, while SDA was selected for its ability to support the growth of dermatophytes and other pathogenic fungi (Watanabe, 2010). Both media were supplemented with streptomycin (500 mg/L) to suppress bacterial contamination.

Isolation of Fungi

The isolation of fungi was carried out following the method of Onuh *et al.* (2015). Cocoyam tubers showing visible spoilage symptoms were first surface sterilised by immersion in 40% sodium hypochlorite solution for 60 seconds and rinsed three times in sterile distilled water. Sections (5 × 5 mm) containing both diseased tissue and advancing margins were excised aseptically and inoculated onto PDA and SDA plates. Inoculated plates were sealed with parafilm and incubated at 28 °C for 72 hours. Emerging fungal colonies were sub-cultured repeatedly on fresh PDA and SDA until pure cultures were obtained.

Identification and Characterisation of Isolates

Fungal isolates were characterised based on cultural,morphological, and microscopic features using standard identification keys (Nwachukwu & Osuji, 2008; De Hoog *et al.*, 2020).

Ethanol Extraction

Fresh leaves of *Vernonia amygdalina* and *Hyptis suaveolens* were sliced into small pieces and air-dried at room temperature (27 ± 2°C) for fifteen (15) days until a constant weight was obtained. The dried samples were ground into fine powder using a sterilized electric grinder and stored in airtight containers until further use.

Ethanol extraction was carried out by cold maceration following the procedure of Ebana *et al.* (2016). A portion of 250 g of powdered plant material was weighed into a sterile conical flask and immersed in absolute ethanol until the material was fully submerged. The flask was tightly sealed with a glass stopper and intermittently agitated every four hours (except at night) for seventy-two (72) hours. At the end of the maceration period, the contents were filtered sequentially through sterile muslin cloth and Whatman No. 1 filter paper. The marc (residual plant material) was pressed to maximize recovery of the extract. The filtrate was concentrated

under reduced pressure at 55°C using a rotary evaporator to obtain the crude ethanol extract. The dried extracts were weighed, stored in sterile airtight containers, and refrigerated at 4°C until further analysis.

Qualitative Phytochemical Screening

Phytochemical screening of the plant extracts was conducted to detect the presence of bioactive secondary metabolites such as alkaloids, phenolics, terpenoids, cardiac glycosides, flavonoids, saponins, steroids, and tannins. The procedures described by Banso and Adeyemo (2019) were adopted with slight modifications.

Antifungal Activity

The antifungal activity of the plant extracts against fungal isolates obtained from spoilt cocoyam was evaluated using the agar well diffusion method as described by CLSI (2012) and modified by Ohikhena *et al.* (2017).

Agar Well Diffusion Assay

Fungal suspensions were standardised to a concentration of approximately 2.5×10^4 CFU/mL using sterile saline. A 100 µL aliquot of the fungal suspension was evenly spread onto the surface of freshly prepared SDA plates using sterile cotton swabs. The plates were allowed to stand for 15 minutes to ensure proper adherence of inocula.

Sterile cork borers (6 mm diameter) were used to punch wells into the agar. Each well was filled with 50 µL of plant extract at concentrations of 25%, 50%, and 100%. Fluconazole (10

mg/mL) served as the positive control, while sterile distilled water was used as the negative control.

The inoculated plates were pre-incubated at 4 °C for 2 hours to allow proper diffusion of extracts, followed by incubation at 28 ± 2 °C for 5 days. Antifungal activity was determined by measuring the zone of inhibition (mm) around each well with a transparent ruler. All experiments were conducted in triplicate, and the mean ± SEM values were recorded (Ebena *et al.*, 2016).

0% inhibition = Not effective

1-19% inhibition = Slightly effective

20-49% inhibition = Moderatly effective

50-99% inhibition = Effective

100% inhibition = Highly effective

Results

Table 4. 1: Morphological characteristics of fungal isolates from rotted cocoyam

Isolate code	**Spore colour**	**Agar reverse colour**	**Aerial hyphae**	**Abundance**	**Growth rate**	**Pigmentation**
1	Blue-green	Cream	Powdery, embedded spores	Abundant	Fast	Absent
2	Black	Light green	Powdery, embedded spores	Abundant	Fast	Absent

3	White	Cream	Fluffy, not raised	Abundant	Fast	Absent
4	White	Cream	Fluffy, slightly raised	Abundant	Fast	Absent
5	Black	Light green	Powdery, embedded spores	Abundant	Fast	Absent

Based on these characteristics, the following fungal species were identified:

- *Aspergillus flavus*
- *Aspergillus niger*
- *Fusarium solani*
- *Rhizopus stolonifer*
- *Penicillium expansum*

Table 4.2: Phenotypic and microscopic properties of fungal isolates from rotted cocoyam

Isolate code	**Description**	**Probable identity**
1	Colonies smoke-grey in dark, yellowish brown in light; aromatic odour; thick-walled sporangiophores with granular contents.	*Rhizopus stolonifer*
2	Colonies blue-green with columnar conidial heads; pigmented conidiophores with clavate vesicles.	*Aspergillus flavus*

3	Colonies fast-growing, floccose, white to peach with violet tinge; aromatic odour reminiscent of lilac.	*Fusarium solani*
4	Colonies light green, reverse colourless; branched conidiophores; sub-globose conidia; fruity odour.	*Penicillium expansum*
5	Colonies powdery black; conidiophores thick-walled, radiating heads with rough globose conidia.	*Aspergillus niger*

This table confirms fungal identity of *R. stolonifer, A. flavus, F.solani, P. expansum* and *A.niger* using morphological and structural traits viewed under a microsco

Table 4.3: Qualitative phytochemical composition of *Veronia amygdalina* and Hyptis suaveolens ethanol extract.

Parameters	***Veronia amygdalina***	**Hyptis suaveolens**
Saponin	+++	++
Flavonoid	-	-
Alkaloid	++	-
Tannin	+	+
Steroids	-	-
Tepernoid	++	-
Glycosid	+	++
Anthrocynin	+++	-
Phenol	+++	-
Oil and Resin	-	+

Key

+++ = Present in high concentration

++= Present in moderate concentration

+ = Slightly or sparingly present

- = Absent

Table 4.4: Zones of Inhibition of the Extracts against the Isolated Fungi

EXTRACTS	***Aspergillus nigea***	***Penicillium expansum***	***Aspergillus flavus***	***Fusarium solani***	***Rhizopus stolonifer***
***Veronia amygdalina* extract @100%**	20.10±1.00	21.10±0.02	30.00±2.00	17.00±3.01	20.10±0.55
***Veronia amygdalina* extract @ 50%**	11.10±0.01	20.00± 0.06	24.50±1.0^{g}	10.50±1.110	17.00±0.10
***Veronia amygdalina* extract @ 25%**	8.00±0.30	10.50±0.60	19.00±0.20	7.00± 2.00	10.00±0.05

***Hyptis suaveolens* extract @100%**	13.67±1.00	0.00±0.00	24.70±2.00	12.20±3.01	7.60±0.13
***Hyptis suaveolens* extract @50%**	7.00±0.01	0.00±0.00	22.20±1.00	7.50±1.10	0.00±0.00
***Hyptis suaveolens* extract @25%**	5.18±0.30	0.00± 0.00	15.86±0.20	6.70± 2.00	0.00±0.00
Fluconazole 30µg/ml	22.33±0.11	34.833±0.15	41.60±1.00	34.83± 0.3	19.16±1.00

*Values are mean scores ± Standard deviation of three (3) replicates

N/B: CLSI specification and standard zone of inhibition ranges:

Less than or equal to 15mm = microbes are resistant to the extract

16 - 20 = Microbes are intermediate to the extract.

21 and above = Microbes are susceptible to the extract.

- *V. amygdalina* showed the highest inhibition across all test fungi, except for *F. solani*, suggesting its broad-spectrum antifungal potential.
- *Hyptis suaveolens* exhibited the weakest antifungal activity among the two extracts

Discussion

The fungus found in the decomposing cocoyam corms purchased in the main markets of Anambra state were *Aspergillus niger, Fusarium solani, Penicillium expansum, Rhizopus stolonifer and Aspergillus flavus*. This was in conformity with previous publications (Okigbo and Nwatu, 2015; Ewekeye *et al.*, 2017) which often links them to the post harvest degradation of cocoyam and other tropical tuber crops.

The result of the study corroborates the findings of studies done in Nigerian markets and related tropical store. Previous studies have revealed the presence of *Aspergillus spp.*, and *Rhizopus spp*., on cocoyam in local markets; similarly, the occurrence of *A. niger* and *R. stonifera*

Other scientists have reported Stolonifer on similar samples (Ewekeye *et al.*, 2017; 2020; Chukwuka *et al*., 2020).

These findings are along public health lines, and must not be ignored. The detection of some of the isolates obtained in this study that are capable of producing mycotoxins in marketed cocoyam, also suggests other factors apart from physical deterioration (Okigbo *et al.*, 2018).

The in vitro antifungal test indicated that ethanolic extracts of the five plants prevented growth of all of the isolated spoilage fungi, but the extent of growth inhibition depended on the fungus type and concentrations. The dose-dependent relationship was observed because generally the inhibition zones increased from 25% extract concentration to 50% and 100% extract concentration.

Other previous antifungal studies with plant extracts have shown similar antifungal effects at different concentrations (De Lange *et al.*, 2018; Iwu, 2017). The botanicals were actually able to achieve some antifungal activity as several of the extracts were equally active against some of the fungus with fluconazole being the most effective overall.

Vernonia amygdalina was the best overall inhibitor among the two plants tested, especially for *Rhizopus stolonifer, Aspergillus flavus* and *Penicillium expansum*. Phytochemical analysis revealed the presence alkaloids,terpenoids, flavonoids,tannins and that of phenolics and saponins which may be related to the better performance as phenolics commonly inhibit enzyme function and saponins disrupt fungal membranes. On the other hand, *Hyptis suaveolens* yielded the lowest inhibitory zones against most of the test fungus. The latter could be because of less extraction of the metabolites of interest under the study conditions or decreased levels of specific bioactive chemicals. The flavonoids and phenolics related chemicals in the extracted may be correlated with that effect. The polyphagic activity observed during this research aligns with the secondary metabolites reported extensively as antimicrobial agents such as alkaloids, flavonoids, tannins and saponins (Okonkwo and Ogu, 2019; Ejimofor and Oledibe, 2022).

Although fluconazole gave greater zone of inhibition, the plant extracts did not differ by much to suggest that when improved or prepared properly natural alternatives may be beneficial. So, the results corroborate ethnobotanical uses of these plants for post harvest deterioration and associated plant diseases control. Furthermore, they show that the plants herbes that can be found locally have a potential use as a source for developing safer anti-fungal drugs.

Conclusion

The extracts exhibited important phytochemical composition and bioactive compounds that include flavonoids, alkaloids,terpenoids,tannins, and that of phenolics and saponins which could be correlated with the reported inhibitory effects.

Vernonia amygdalina and Hyptis suaveolens ethanol extracts were shown to be fungus growth inhibitors in the present research. The most effective fungitoxic effects were caused V. amygdalina while *H. suaveolens* was not as active as compared under the study condition.

Results indicate that some plant extracts may have a potential to be employed as substitutes to the chemical fungicide; benefits of this can include reducing dependence on synthetic fungicides, lower cost of preservation and exploitation of locally available botanical material.

Therefore, the results provide scientific evidence in favour of the traditional usage of these plants for crop protection and indicate that they could be helpful for sustainable cocoyam postharvest management.

Recommendation

Pharmacological and toxicological studies are suggested to be carried out to assure that plant extracts are safe and to determine the proper concentration to be used in real life.

Plant products as fungicides: Further research is needed to isolate, characterize and synthesize these plant extract active components and us them in making commercial fungicides.

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