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Antifungal Potential of Ethanol Leaf Extract of Bitter Leaf (*Vernonia Amygdalina* L) Against *Dioscorea rotundata* (White Yam) Spoilage Fungi

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Abstract

Dioscorea rotundata is one of the most cherished yam varieties, but it is unfortunately highly susceptible to fungal infection, posing a significant threat to food security. Efforts to address this issue with conventional fungicides have worsened the situation by compromising food safety. This study aimed to assess the potential of *Vernonia amygdalina* extract to protect yam tubers against fungal attack. Freshly harvested leaves of *Vernonia amygdalina* (bitter leaf) from a household garden were processed into a fine powder. Exactly 500 g of *Vernonia amygdalina* ethanol leaf extract (VAELE) was macerated in 1,000 mL of 70% ethanol for 72 hours before being filtered. The resulting filtrate was concentrated using a rotary evaporator. Microbial procedures and analyses, such as fungal isolation, identification, anti-fungal sensitivity bioassay, minimum inhibitory concentration (MIC), and minimum fungicidal concentration, were performed using standard procedures. Fungal presence was most pronounced on the 6th (45.0 × 10⁶) and 7th spots (55.0 × 10⁶ CFU/g) SYTS. However, the 5th spoiled yam tuber spot (SYTS) manifested the least fungal load of 3.0 × 10⁶ CFU/g. *Aspergillus niger* recorded the highest occurrence (34.42%), whereas *Fusarium oxysporum* had the lowest (14.75%). The anti-fungal activity of VAELE was dose-dependent, with the most pronounced activity observed at 300 mg/mL. The minimum inhibitory concentration (MIC) was established at 75 mg/mL against all fungal isolates (*A. niger*, *A. flavus*, *P. chrysogenum*, and *F. oxysporum*). Fungicidal activity of VAELE was established at 300 and 150 mg/mL. In conclusion, it can be deduced from this study that *Vernonia amygdalina* ethanol leaf extract can impede or kill *A. niger*, *A. flavus*, *P. chrysogenum*, and *F. oxysporum* depending on the concentration applied.

Keywords: *Vernonia amygdalina*; *Aspergillus niger*; *Dioscorea rotundata*; Fungicidal; *Aspergillus flavus*

Received: 3rd February 2026 / Accepted: 11th April 2026 / **Published online:**30th April 2026

© The Author(s), under exclusive license to Bio-Research-Journal-of-Biological-Research-and-Biotechnology (JBRB), ISSN: 2705-3822; print-ISSN: 1596-7409 (Online); Open Access This article is available at <https://bio-research.com.ng/index.php/home>, 2026.

DOI: <https://dx.doi.org/10.4314/br.v24i1.14>

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INTRODUCTION

Yams, an edible tuber belonging to the genus *Dioscorea* (family *Dioscoreaceae*), are a major staple crop in tropical regions, providing substantial dietary energy and supporting the livelihoods of millions of people worldwide. Globally, it is one of the most widely cultivated tuber crops, with West Africa contributing the majority of production, and Nigeria recognized as a leading producer in the region (FAO, 2023; IITA, 2022). In addition to being a dependable source of carbohydrates, yams provide several micronutrients with health benefits, including vitamins such as thiamine, riboflavin, niacin, ascorbic acid, and carotene, although they should not be relied upon as a sole source of these micronutrients. They also contain essential amino acids such as arginine, leucine, isoleucine, and valine, along with smaller amounts of methionine, tryptophan, and histidine (Mondo et al., 2022). *Dioscorea rotundata* (white yam) is the most widely cultivated and consumed yam species in Nigeria. It is valued for its palatability as well as its social, cultural, and economic importance; however, it is highly susceptible to post-harvest fungal invasion, particularly during storage, where spoilage typically becomes evident within 2–3 months under suboptimal conditions (Gwa & Ekafan, 2021). This susceptibility contributes significantly to post-harvest losses and reduced market value. Although synthetic fungicides have been employed in managing post-harvest yam spoilage, their use is associated with notable drawbacks, including toxicity to non-target organisms, environmental contamination, and the emergence of resistant fungal strains (Ezebo & Okigbo, 2024). These limitations have intensified interest in alternative control strategies. Plant-based options refer to the use of whole plants, plant parts, or crude extracts with inherent antimicrobial properties, such as neem (*Azadirachta indica*) leaf extract, garlic (*Allium sativum*) extract, and ginger (*Zingiber officinale*) extract. These are often used directly in crude or minimally processed forms. In contrast, plant-derived products are more refined preparations obtained from plants, including essential oils (e.g., clove oil from *Syzygium aromaticum*), plant-derived bioactive compounds (e.g., allicin from garlic), and formulated botanical pesticides such as neem-based commercial products. These plant-based and plant-derived approaches are generally biodegradable, locally accessible, and widely regarded as environmentally sustainable alternatives to synthetic fungicides (Isman, 2020).

Vernonia amygdalina Del., commonly known as bitter leaf, is a perennial shrub in the family Asteraceae that is widely distributed in tropical regions. It has long been used in traditional medicine for the treatment of malaria, helminth infections, and various gastrointestinal and inflammatory conditions. Recent studies have reported that its antimicrobial activity is associated with bioactive phytochemicals such as flavonoids, saponins, alkaloids, and terpenoids, which exhibit antifungal, antibacterial, and antiparasitic properties (Degu et al., 2024).

While several studies have demonstrated the antifungal activity of *V. amygdalina* against human and plant-associated

pathogens, there remains limited empirical evidence evaluating its efficacy against fungal pathogens responsible for yam tuber spoilage. Furthermore, most existing studies have focused on in vitro antimicrobial screening without adequately addressing its effectiveness under conditions that mimic post-harvest storage environments of yam tubers. This highlights a critical knowledge gap in understanding its practical application as a bio-preservative in yam storage systems.

Therefore, this study seeks to evaluate the antifungal potential of *Vernonia amygdalina* extract against fungi associated with yam spoilage, to contribute to the development of sustainable and plant-based post-harvest protection strategies.

Materials and methods

Sample collection

Fresh leaves of bitter leaf (*Vernonia amygdalina*) were harvested from a garden located within the Federal College of Forestry, Jos (9.8946° N, 8.8580° E). The leaves were subsequently taken to the college herbarium in a polyethylene bag for identification. Four infected and four uninfected yam tubers were procured from Yandoya market in Jos North, Plateau State, and placed in separate polyethylene bags labeled appropriately before being taken to the Biology Laboratory at the Federal College of Forestry for further study.

Plant extract preparation

Fresh leaves of *Vernonia amygdalina* were collected, washed thoroughly with clean tap water to remove debris, and air-dried at room temperature (28 ± 2 °C) until a constant weight was achieved. The dried leaves were pulverized into a fine powder using a mechanical grinder and sieved through a wire mesh (425 µm) to obtain a uniform particle size. Precisely 200 g of the powdered plant material was introduced into a conical flask containing 500 mL of 70% ethanol. The mixture was macerated for 72 hours with intermittent stirring to enhance the extraction. After maceration, the mixture was filtered using a Whatman No. 1 filter paper muslin cloth, and the remaining plant residue was rinsed several times with fresh solvent to ensure maximum recovery of extractable components. The combined filtrate was concentrated to dryness using a rotary evaporator under reduced pressure. Eighty (80) g of dry extract was carefully transferred into a sterile, airtight sample container and stored in a refrigerator at 4 °C until further use. The percentage yield of the extract (40%) was calculated following the equation below (Harborne, 1998).

$$\% \text{ yield} = \frac{\text{Weight of dried extract obtained (g)}}{\text{Weight of initial dried plant material (g)}} \times 100 \dots \dots (1)$$

Preparation of the Medium

<https://dx.doi.org/10.4314/br.v24i1.14>

Exactly 39 g of Potato Dextrose Agar (PDA) was dissolved in 1000 mL of distilled water, heated on a hot plate until fully homogenized, and sterilized by autoclaving at 121 °C for 15 minutes. After autoclaving, the medium was allowed to cool to approximately 45 °C, after which 2 mL of 10 mg/mL gentamicin was aseptically added to suppress bacterial growth. The mixture was gently swirled to ensure uniform distribution of the antibiotic, then poured into sterile Petri dishes and allowed to solidify (Atlas, 2010).

Isolation of fungi

The infected portion of the yam tuber was excised and surface-sterilized by immersion in 75% ethanol for 60 seconds, followed by thorough rinsing in sterile distilled water. The tissue was then chopped into small pieces and blot-dried using Whatman No. 1 filter paper. The dried pieces were aseptically inoculated onto Petri dishes containing pre-solidified potato dextrose agar (PDA). The plates were incubated in the dark at room temperature (28 ± 2°C) and monitored daily for fungal growth. Fungal colonies that emerged were sub-cultured onto fresh PDA plates to obtain pure cultures (Fawole & Oso, 2012).

Fungi identification

Using a sterile inoculating needle, small portions of the actively growing edge of each colony were transferred onto a clean, grease-free glass slide. Two drops of lactophenol cotton blue were added, after which the preparation was covered with a coverslip to prevent dehydration. The slide was examined under a compound microscope at 10× and 40× magnifications. Fungal morphology, including the appearance of conidia and conidiophores, was identified by comparing the observed structures with descriptions provided by Barnett & Hunter (1998). The percentage frequency occurrence of each fungus was calculated using the following formula:

$$\% \text{ occurrence} = \frac{\text{Number of individual fungi isolates}}{\text{Total number of fungi isolates}} \times 100 \dots (2)$$

Antifungal sensitivity bioassay

In vitro antifungal sensitivity testing was carried out using the poisoned food technique following the method of Sangoyomi (2004). Stock extract solutions were prepared at concentrations of 5%, 10%, 15%, and 20%. For each treatment, 1 mL of the respective stock extract was dispensed into sterile Petri dishes, followed by the addition of 9 mL of molten Potato Dextrose Agar (PDA) to obtain final extract concentrations of 2.5%, 5.0%, 7.5%, and 10%. The plates were gently swirled to ensure uniform mixing and allowed to solidify. Each plate was centrally inoculated with a 6 mm mycelial disc obtained from the actively growing margin of a 4-day-old culture of the test fungus. Extract-free PDA plates inoculated with the test fungi served as the control. All treatments were conducted in triplicate. The plates were incubated at 27 °C, and radial mycelial growth was measured daily for five days. Colony diameter was determined as the

mean of two perpendicular measurements taken along pre-marked lines on the underside of each plate. Percentage inhibition of mycelial growth was calculated according to Whipps (1987) using the formula:

$$\text{inhibition of mycelial growth} = \frac{R1-R2}{R1} \times 100 \dots (3)$$

Where R1 = is the furthest radial distance of pathogens in the control plates; Where R2 = is the furthest radial distance of pathogens in the extract (treated) plates.

Minimal inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) determination was performed using the broth microdilution (two-fold serial dilution) method as described by Fabry et al. (1998). Crude extract stock solutions were prepared in 10% (v/v) dimethyl sulphoxide (DMSO), with the final DMSO concentration in the assay not exceeding 1% (v/v), and serial two-fold dilutions were made to obtain final concentrations of 300, 150, 75, 37.5, and 18.7 µg/mL. Each concentration was dispensed into sterile broth medium (e.g., potato dextrose broth) contained in test tubes and inoculated with standardized fungal spore suspensions (≈10⁶ spores/mL) of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, and *Fusarium oxysporum*. All tubes were incubated at 28 ± 2 °C for 48 hours. Fungal growth was assessed visually and further quantified by measuring optical density at 630 nm. The MIC was defined as the lowest extract concentration that showed no visible fungal growth. Percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{OD of ctrl} - \text{OD of test}}{\text{OD of ctrl}} \times 100 \dots (4)$$

Determination of minimum fungicidal concentration (MFC)

The minimum fungicidal concentration (MFC) of the ethanol leaf extract of *Vernonia amygdalina* against fungi isolated from spoiled *Dioscorea rotundata* was determined using the microdilution-subculture technique as described by CLSI (2017) and Balouiri et al. (2016), with slight modifications. After 72 hours of incubation, 20 µL aliquots were aseptically withdrawn from microdilution wells that showed complete inhibition of fungal growth (optically clear wells), as well as from the last well showing visible growth and the extract-free growth control well. To avoid resuspension of fungal cells, the contents of the wells were not agitated before sampling. Each aliquot was subcultured onto Sabouraud dextrose agar (SDA) plates and incubated at 28 ± 2 °C. The plates were observed for fungal growth, which appeared on growth control plates within 48 hours. The MFC was defined as the lowest extract concentration that produced no visible fungal growth or fewer than three colonies on SDA plates, corresponding to approximately 99.5% fungicidal activity.

Pathogenicity test

Healthy and apparently disease-free yam tubers (*Dioscorea rotundata*) were washed thoroughly under running tap water, rinsed with distilled water, and surface-sterilized with 70% ethanol. Using a sterile cork borer, cylindrical cores approximately 3 cm deep were aseptically removed from different regions of each tuber to create uniform wounds. A 5 mm diameter mycelial disc obtained from an actively growing fungal culture was inserted into each cavity, after which the excised yam tissue was trimmed and replaced to reseal the wound. The inoculation sites were covered with sterile cotton wool lightly coated with petroleum jelly to minimize moisture loss and prevent secondary contamination. Each inoculated tuber was placed individually in a moistened, sterile, airtight polyethylene bag and incubated at room temperature (28 ± 2 °C) for an initial period of 14 days under high-humidity conditions. Disease development was assessed by visual observation of symptom expression and lesion formation on days 14, 21, and 32 post-inoculation. Control tubers were treated similarly, except that sterile potato dextrose agar (PDA) discs without fungal mycelia were inserted into the cavities. All treatments were carried out in replicates, and data were collected according to the same observation schedule.

Data analysis

Data generated from this study were expressed as mean ± error of the mean from three determinations. Mean values were compared using one-way analysis of variance (ANOVA). A statistically significant difference was established at the 95% confidence level ($p < 0.05$).

Results

Fungal loads from spoiled yam tubers

The fungal loads obtained from the spoiled yam tubers are displayed in Table 1, indicating a variation in the extent of fungal contamination. While the presence of fungi was relatively low in some parts of the yam tubers, it was quite high in other parts. The 5th spoiled yam tuber spot (SYTS) presented the least fungal load of 3.0×10^6 CFU/g. The 10th, 1st, and 2nd SYTS presented moderate fungal colonization of 11.0×10^6 CFU/g to 19.0×10^6 CFU/g. However, SYTS 3, 4, 8, and 9 demonstrated elevated fungal presence (40.0×10^6 CFU/g, 32.0×10^6 CFU/g, 35.0×10^6 CFU/g, and 43.3×10^6 CFU/g), respectively. Extremely high (45.0×10^6 and 55.0×10^6 CFU/g, respectively) fungal activity was noticed in the 6th and 7th SYTS.

Table 1: Total viable count (CFU/g) of fungi isolated from spoiled yam tubers

Spots	Fungi loads (CFU/g)
1	11.0 X 10 ⁶
2	12.0 X 10 ⁶
3	40.0 X 10 ⁶
4	32.0 X 10 ⁶
5	3.0 X 10 ⁶
6	45.0 X 10 ⁶
7	55.0 X 10 ⁶
8	35.0 X 10 ⁶
9	43.3 X 10 ⁶
10	19.0 X 10 ⁶

Percentage occurrence of isolates

The results in Figure 1 show the percentage occurrence of the fungal isolates. The percentage occurrence and number of isolates recorded for *F. oxysporium* were smaller than those of *P. chrysogenus* which is smaller than those of *A. flavus* which in turn is lower than those of *A. niger*. Among all the isolates, *Aspergillus niger* recorded the highest occurrence (34.42%) as well as number of isolates, whereas *Fusarium oxysporum* had the lowest (14.75%).

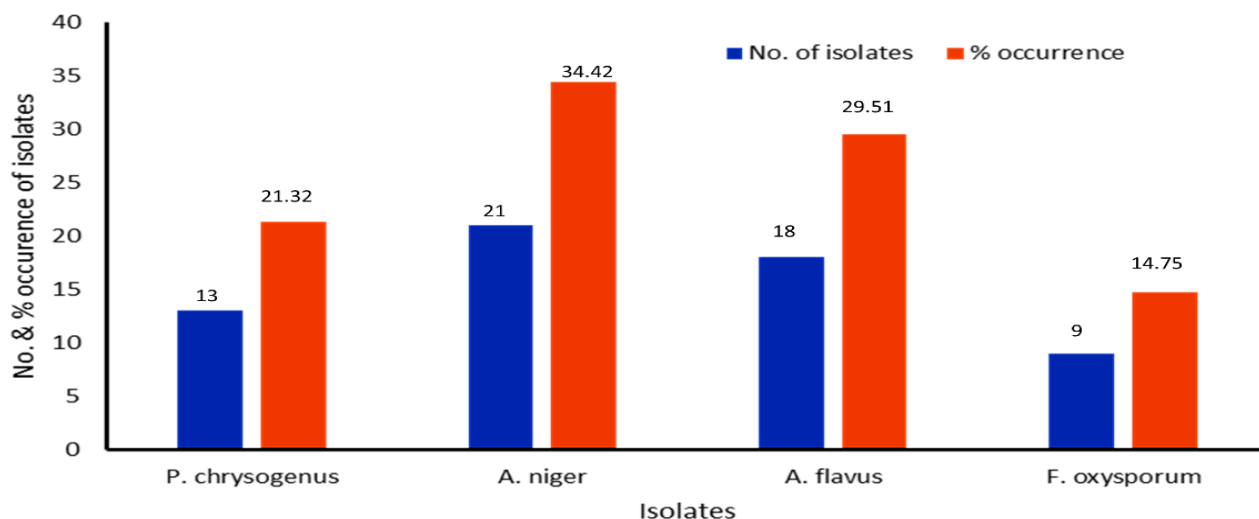


Figure 1: Percentage occurrence of isolates

Pathogenicity assay

The results presented in Table 2 show the mean pathogenicity effects (mm) of the fungal isolates. A significant difference ($p < 0.05$) was observed among the isolates. *Penicillium chrysogenum* exhibited a significantly higher ($p < 0.05$) pathogenicity value than *Fusarium oxysporum*, which, in turn, did not differ significantly ($p < 0.05$) from *Aspergillus flavus*. However, the value for *F. oxysporum* was significantly ($p < 0.05$) higher than that recorded for *Aspergillus niger*.

Anti-fungal activity of ethanol leaf extract of bitter leaf on fungal isolates

The antifungal activity of the ethanol leaf extract of bitter leaf (*Vernonia amygdalina*) against fungal isolates from spoiled yams is presented in Table 3. The results show a clear dose-dependent response. At 300 mg/mL, the extract exhibited significantly higher ($p < 0.05$) antifungal activity against *A. niger*, *A. flavus*, *P. chrysogenum*, and *F. oxysporum* than at 150 mg/mL. Similarly, the inhibition recorded at 150 mg/mL was significantly ($p < 0.05$) greater than that at 37.5 mg/mL, which in turn exceeded the activity at 18.7 mg/mL. As expected, the standard drug showed the highest overall antifungal activity.

Table 2: Comparative pathogenicity of selected fungal isolates based on lesion development

Isolate	Lesion size (mm)
<i>Aspergillus niger</i>	0.45±0.47 ^a
<i>Aspergillus flavus</i>	2.18±2.87 ^{bc}
<i>Penicillium chrysogenum</i>	3.31±2.77 ^d
<i>Fusarium oxysporum</i>	2.30±4.07 ^{bc}

Results are expressed as mean ± standard deviation of three determinations. Values in the same column bearing the same subscript letters do not differ significantly at $p > 0.05$.

Table 3: Anti-fungal activity of bitter leaf (*Vernonia amygdalina*) ethanol leaf extract on fungal isolates from spoiled yam tubers

Conc. (mg/mL)	<i>A. niger</i>	<i>A. flavus</i>	<i>P. chrysogenum</i>	<i>F. oxysporum</i>
300	17.30±0.56 ^e	22.20±1.56 ^e	37.86±2.72 ^f	25.23±0.51 ^e
150	16.93±0.59 ^d	18.33±2.57 ^d	26.00±2.57 ^d	22.70±0.27 ^d
75	14.60±0.70 ^c	17.10±2.17 ^c	23.00±1.56 ^c	21.03±1.46 ^c
37.5	13.70±0.67 ^b	14.00±1.52 ^b	18.36±2.56 ^b	19.73±1.96 ^b
18.7	0.30±0.16 ^a	10.70±1.69 ^a	15.86±2.56 ^a	15.96±3.11 ^a
Fluconazole (10 mg/mL)	34.30±0.56 ^f	24.83±2.66 ^f	28.96±1.86 ^e	28.86±2.22 ^d

Results are expressed as mean ± standard error of the mean (SEM) of three determinations. Values in the same column bearing the same subscript letters do not differ significantly at $p < 0.05$.

Minimum inhibitory concentration (MIC) of bitter leaf extract on fungi isolates from spoiled yam tubers

The results in Table 4 revealed the Minimum Inhibitory Concentration (MIC) of the ethanol leaf extract of *V. amygdalina* on fungi isolates from spoiled yam tubers. The results showed that the isolates were inhibited using 300, 150, and 75 mg/mL of the plant extract. The minimum inhibitory concentration of the bitter leaf extract against the isolates was determined to be 75 mg/mL.

Minimum Fungicidal Concentration (MFC) of Bitter Leaf Extract on fungi isolates from spoiled yam tubers

The minimum fungicidal concentration (MFC) of the ethanol leaf extract of *Vernonia amygdalina* against fungal isolates from spoiled yam tubers is presented in Table 5. The results indicate that an MFC of 150 mg/mL was obtained for *Aspergillus niger* and *Aspergillus flavus*, whereas a lower MFC of 75 mg/mL was recorded for *Penicillium chrysogenum* and *Fusarium oxysporum*. Overall, the extract demonstrated fungicidal activity against all tested isolates at concentrations of 300 mg/mL and 150 mg/mL, indicating a concentration-dependent effect

Table 4: Minimum inhibitory concentration (MIC) of bitter leaf extract on fungi isolates from spoiled yam tubers

Isolates	Concentrations (mg/mL)					MIC
	300	150	75	37.5	18.7	
<i>Aspergillus niger</i>	-	-	-	+	+	75
<i>Aspergillus flavus</i>	-	-	-	+	+	75
<i>Penicillium chrysogenum</i>	-	-	-	+	+	75
<i>Fusarium oxysporum</i>	-	-	-	+	+	75
Ethanol	-	-	-	-	-	-

Key: = + (no inhibition), - (inhibition)

Table 5: Minimum fungicidal concentration (MFC) of bitter leaf extract on fungi isolates from spoiled yam tubers

Isolates	Concentrations (mg/mL)					MFC
	300	150	75	37	18.7	
<i>Aspergillus niger</i>	+	+	-	-	-	150
<i>Aspergillus flavus</i>	+	+	-	-	-	150
<i>Penicillium chrysogenum</i>	+	+	+	-	-	75
<i>Fusarium oxysporum</i>	+	+	+	-	-	75

Key: = + (growth absent), - (growth present)

Discussion

The role of fungi in postharvest yam spoilage cannot be overemphasized. The variation in fungal counts observed at different portions of the yam tubers could be attributed to the variation in nutrient density across the tuber. This is in tandem with the outcome of a study by Sila et al. (2024), which indicated that glucose mobilization, as well as transportation from the inner layers to the peel by the migration of water content, enhanced the ability of fungi to destroy stored potatoes. Fungal load as high as 55.0×10^6 CFU/g suggests severe tissue breakdown and raises significant food safety concerns due to the possibility of mycotoxin production from *Aspergillus* species (Navale et al., 2021). The observed high prevalence of *A. niger* could be attributed to *A. niger*'s ability to thrive in diverse environmental conditions, reproduce rapidly, and withstand low moisture levels (Rabab et al., 2025). Pathogenicity tests revealed a wide variation in the pathogenicity of the fungal isolates. *P. chrysogenum* was the most pathogenic, likely due to its ability to produce enzymes that can degrade yam tissue more rapidly. The high pathogenicity score associated with *Penicillium chrysogenum* could be attributed to *P. chrysogenum*'s ability to synthesize enzymes that can break down the host cell membrane, thereby making invasion easier. This is in tandem with the outcome of a study by Yassein (2020), which established the pathogenicity of *P. chrysogenum* in mice. Studies on the antifungal potential of VAELE demonstrated an explicit dose-dependent inhibitory effect against all isolates. Greater zones of inhibition were produced with higher concentrations of VAELE, an indication of stronger antifungal activity. This finding is in tandem with the outcome of a study by Ijeh & Ejike (2011), which showed that *V. amygdalina*'s broad-spectrum antimicrobial activity is attributed to its inherent phytochemicals such as saponins, flavonoids, alkaloids, tannins, and sesquiterpene lactones. The fungistatic activity of *V. amygdalina* is revealed through the minimum inhibitory concentration of 75 mg/mL established against all isolates. Similarly, the minimum fungicidal concentration showed that VAELE was fungicidal against *P. chrysogenum* and *F. oxysporum* at 75 mg/mL. However, higher concentrations were needed to elicit a fungicidal effect against *A. niger* and *A. flavus*. The observed variation in susceptibility could be a result of the differences in fungal cell wall composition and resistance mechanism (Cowan, 1999).

Conclusion

The findings of this study indicate that spoilage of *Dioscorea rotundata* is predominantly associated with pathogenic fungi, particularly *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, and *Fusarium oxysporum*. The ethanol leaf extract of *Vernonia amygdalina* demonstrated dose-dependent antifungal activity against all isolated fungi, as reflected by the relatively low minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values obtained. These results suggest that the extract possesses promising antifungal potential and may serve as a viable botanical agent for the preservation of harvested *D. rotundata*, thereby contributing to improved post-harvest management and food security. However, while the extract exhibited inhibitory and putative fungicidal effects, these findings remain preliminary. Further investigation is therefore necessary to substantiate its fungicidal efficacy, particularly through time-kill assay studies to elucidate the rate and extent of fungal inactivation over time. Such studies will provide deeper insight into the mechanism of action and practical applicability of the extract under real storage conditions.

Conflict of interest

Authors hereby declare that no conflict of interest exists

Acknowledgements

Authors sincerely appreciate the roles played by the technical staff of the Department of microbiology, Federal College of Forestry Jos, in ensuring the timely completion of this study.

Authors' contributions

JWC conceptualized the problem and designed the experiment, **MA** performed the experiment, while **RMK** conducted the statistical analysis and proofread the manuscript prior to submission.

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