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Original Article

# Determination of Antifungal and Phytochemical Properties of Common Hydrophytes in Nigeria: Insight on Post-Harvest Fungal Pathogens of Fruit Pulp of *Phoenix dactylifera*

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## Abstract

This study evaluated the antifungal and phytochemical properties of three common hydrophytes (*Nymphaea lotus*, *Paspalum scrobiculatum*, and *Eichhornia crassipes*) in Makurdi, Nigeria, focusing on their efficacy against post-harvest fungal pathogens. The hydrophyte extracts were screened for phytochemical content and tested against fungal isolates (*Fusarium oxysporium*, *Rhizopus nigricans*, *Mucor indicus*, and *Aspergillus niger*) from *Phoenix dactylifera* using the petroleum ether and water extraction methods. Results indicated that *Eichhornia crassipes* exhibited the strongest antifungal activity, with inhibition zones ranging from 15 mm to 19 mm and the lowest minimum inhibitory concentration values (12.50-25.00 mg/mL). *Nymphaea lotus* showed moderate activity, while *Paspalum scrobiculatum* had the weakest effect. Phytochemical analysis revealed the presence of tannins, phenols, flavonoids, terpenoids, steroids, and cardiac glycosides in all extracts, with *E. crassipes* containing the highest concentrations of tannins (2.65 mg/g), steroids (2.76 mg/g), and phenols (2.30 mg/g). The study concluded that the selected hydrophytes, particularly *E. crassipes*, possess significant antifungal properties that correlates with their rich phytochemical composition. Furthermore, a comprehensive ecological and cytogenetic study of the invasive species in the present work is recommended for future investigation particularly in the production of eco-friendly bio-fungicides suitable for the management of post-harvest crop losses.

**Categories:** Food security and sustainability, Field & Horticultural crops sustainable production, Plant-pathogen interaction

**Keywords:** hydrophytes, post-harvest, antifungal activities, phytohemicals, fungal pathogens

## Introduction

Hydrophytes are plants that live wholly or partly in water. They play critical ecological and socio-economic roles in freshwater ecosystems. They influence nutrient cycling, provide habitat and shelter for aquatic fauna, and are used by local communities for fodder, crafts, and traditional medicine. Several widely distributed hydrophytes (e.g., *Eichhornia crassipes*, *Pistia stratiotes*, *Nymphaea lotus*, and duckweeds) are known to biosynthesize a diversity of secondary metabolites (phenolics, flavonoids, alkaloids, saponins, tannins, glycosides) with potential biological activities [1].

Plants have long served mankind as sources of medicinal agents. These have been found to possess fungicidal properties against various phytopathogenic fungi [2]. Extracts of *Prosopis africana* and *Anacardium occidentale* have been reported to significantly reduce the incidence and the development of *Macrophomina phaseolina* root rot disease both in pre-yield and yield parameters [3]. Similarly, pulverized bark of *P. africana* and leaves of *Nymphia latifolia* inhibited both radial mycelia growth and sclerotia formation of *M. phaseolina* by 100% [4]. Petroleum ether extract and ethanolic extracts of

*Anacardium occidentale* leaves exhibited significant antimicrobial and antifungal activity [5]. Similarly, almost all parts of *P. africana* tree (leaves, stem, and roots) are used in medicine; the leaves in particular are used for the treatment of headache and toothache as well as various other head ailments [6].

In Nigeria, ethnobotanical and pharmacological studies have repeatedly shown that terrestrial and aquatic plants are sources of antimicrobial agents used in traditional healthcare and as leads for drug discovery. Studies of Nigerian plants have documented broad-spectrum antibacterial and antifungal properties that correlate with the presence of specific phytochemical classes, supporting the search for plant-based antifungals to address fungal pathogens affecting humans, animals and crops [7]. Locally available hydrophytes are particularly interesting because their unique aquatic environment can influence metabolite profiles [8]. Hence, they are abundant and easy to sample particularly in the wetland areas such as those around Makurdi.

Recent analytical studies on individual hydrophytes (e.g., studies on *E. crassipes, N. lotus*, and *Typha domingensis*) demonstrate the presence of quantifiable phenolics and flavonoids [9-11] and report antimicrobial or biological activities, reinforcing the hypothesis that common aquatic plants contain compounds of antifungal interest [12]. These findings, together with global interest in plant-derived antifungals as alternatives to synthetic fungicides and as complementary therapies for fungal infections, justify a focused investigation of the phytochemical and antifungal profiles of hydrophytes in Makurdi. Fungal pathogens classified in genera such as *Candida*, *Aspergillus*, *Rhizopus*, *Fusarium* and aflatoxin-producing groups represent significant health and economic burdens globally and in Nigeria, affecting human and animal health and causing crop losses and mycotoxin contamination [13]. Conventional antifungal agents and fungicides face challenges including toxicity, environmental persistence, cost, and the emergence of resistant strains. Locally accessible plant-based antifungals could offer safer, affordable, and sustainable alternatives [14]. However, despite the widespread occurrence of hydrophytes and anecdotal traditional uses in Makurdi, there is a paucity of systematic, region-specific phytochemical profiling and antifungal testing of aquatic plants [15].

The aim of the study was to evaluate the antifungal and phytochemical properties of three hydrophytes in Makurdi.

The specific objectives of the study were as follows:

i. Identify and isolate fungal species from storage fruits of *Phoenix dactylifera* varieties.

ii. Evaluate the antifungal properties of leaf extracts of selected hydrophytes (*N. lotus*, *P. scrobiculatum*, and *E. crassipes*) on dried date fruits pulp.

iii. Determine the phytochemical properties of *N. lotus*, *P. scrobiculatum*, and *E. crassipes.*

## Materials And Methods

### Study area

Makurdi is the capital city of Benue State and is located between latitude 7°38' N and 7°50'N and longitude 8°24'E and 8°38'E. In the past; during the pre-colonial era particularly in 1920, the metropolitan city was just a home to Tiv settlements and Jukun fishermen [16]. The capital city represents a Local Government Area (Makurdi LGA) with a population which has grown steadily from a few thousand people to over 300,000 inhabitants. The increase in population is no doubt accompanied by the expansion of the town with increasing pressure on natural resources within its environs. The climatic conditions of the area are characterized by wet and dry seasons dominated by North-East and South-West monsoon. The wet season begins in April and ends in October with average rainfall ranging from 1,140 mm to 1,440 mm [17]. There is extreme rainfall and peak flooding in the months of August and September. There are variable soil types (loamy sands and sandy clay loam) in the area, which are characterized as hydromorphic in the lowlands and Ferrasols in the upland area [17]. The convergence of wind masses is the predominant influencing factor of rainfall within the study area. The dry season or harmattan begins in November and ends in March.

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**Collection and identification of plant materials**

Fresh leaves of *N. lotus*, *P. scrobiculatum*, and *E. crassipes* were collected from three different locations at Joseph Sarwuan Tarka University Makurdi (JOSTUM) in April 2025. The locations included the Bank of River Benue (*P. scrobiculatum* and *E. crassipes*) and the pond behind the TETFUND Research building in JOSTUM (*N. lotus*). Taxonomic identification of the plant samples was authenticated at the Department of Plant Science and Biotechnology, Joseph Sarwuan Tarka University Makurdi. Voucher specimens were preserved in the herbarium collection of the Department of Plant Science and Biotechnology Makurdi. Ten accessions of *P. dactylifera* were collected from the Nigeria Institute of Oil Palm Research. The samples were air-dried and stored before isolation and identification of fungal species.

**Identification and isolation of the fungi pathogens**

*Preparation of Czapek-Dox Agar Medium (CDA)*

The components of the Czapek-dox agar medium (CDA) were as follows: 3.0 g/l of sodium nitrate ($NaNO_3$), 0.01 g/l of ferrous sulphate ($FeSO_4{\cdot}7H_2O$), 1 g/l of di-potassium hydrogen orthophosphate ($K_2HPO_4$), 0.5 g/l of magnesium sulphate ($MgSO_4{\cdot}7H_2O$), 0.05 g/l of potassium chloride (KCl), 30 g of glucose and plain agar, 1,000 $cm^3$ of distilled water, autoclaved at 15 p.s.i. (121°C for 15 minutes). The CDA medium was prepared as follows: 49 g of CDA was measured and dissolved in 1 L of distilled water (1,000 mL), the mixture was gently heated and stirred consistently until the agar began to boil and the suspension dissolved completely. Next the medium was poured into a flask plugged with cotton wool, then sterilized by autoclaving at a pressure of 15 p.s.i for 15 minutes. The medium was left to cool at a temperature ranging from 45°C to 50°C. To prevent further bacterial contamination, a few drops of chlorophenicol were added before pouring the medium into sterile Petri dishes. The sterilized agar medium was poured into sterile Petri dishes and allowed to solidify [18].

*Preparation of Pure Culture*

Date palm fruits were thoroughly washed under running water to eradicate surface dirt and debris. Furthermore, the fruits were soaked in a 1% sodium hypochlorite solution for 5 minutes. The fruits were further rinsed four to five times in distilled water to completely remove the bleach. Using a sterilized blade, each fruit was opened and cut into small pieces ranging from 2 mm to 3 mm. Extracted tissues were aseptically arranged on the solidified CDA in Petri dishes. The Petri plates were sealed with paraffin and incubated upside down to prevent condensation from dropping onto the fungi at a temperature between 25°C and 28°C for 5-7 days. Fungal growth was observed to appear as fuzzy or powdery colonies. Using a sterile inoculating loop, growing hyphae were carefully cut at the very tip and gently placed in the center of a fresh sterile CDA plate to subculture because the first tissue pieces had developed into various fungal species. To ensure a single species grew without contamination, the new plate was incubated under the same conditions to obtain a pure culture [18].

*Inoculation of Test Organism*

In order to determine how plant extracts (*N. lotus*, *P. scrobiculatum*, and *E. crassipes*) affected the fungi's development and sporulation, 2 mm mycelia discs of the test fungus were injected at the center of the plates using CDA-plant extracts. A 2 mm cork-borer was used to remove the mycelia plugs from the edge of the test fungus's actively growing culture. These were positioned in the middle of each plate, upside down. The inoculation plates were incubated at an average temperature of roughly 28°C ± 2°C. Plant extract-free medium plates were used as a control [18].

*Effect of Plant Extract on Mycelium Growth*

Aqueous extracts were used for the assessment of antifungal properties using the poisoned food method. Initially, 60 mm Petri dishes with CDA solid medium supplemented with 20% aqueous extracts of each plant were used to determine the mycelia growths [18]. Next, a 5 mm diameter disc of fungal mycelium from a pure culture that was seven days old was

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added to the center of each Petri plate and monitored. Fluconazole was used as the control devoid of plant extract to assess mycelium growth response. Ultimately, all of the contaminated plates were incubated for seven days at an average temperature of 25°C.

*Extraction of Plant Materials*

The vegetative plant parts (leaves) of *N. lotus, P. scrobiculatum*, and *E. crassipes* comprised the plant materials. They were sun-dried for two weeks, then milled into a consistent powder using an electric blender (Binatone) with product number: 4173540 and kept until needed. Extracts were used for phytochemical analysis to determine secondary metabolites present in the leaves.

*Petroleum Ether Extraction*

Petroleum ether extraction method was used to extract 200 g of *N. lotus, P. scrobiculatum*, and *E. crassipes*. In a sterile conical flask, 450 mL of the solvent was used to dissolve each plant material for 24 hours day at room temperature. Cotton wool was wrapped in aluminium foil and placed over the conical flask. Before filtering, the extracts were stored for approximately 24 hours. A Whatman filter paper No. 42 (125 mm) and cheesecloth were used in a comprehensive filtration process. Filtrates ranging from dark brown to light brown were collected and put into a specimen vial with a clear label. After the plant waste was powdered, it was spread out on a lab bench and allowed to completely dry for two weeks [18].

*Water Extraction*

Following multiple rounds of distilled water washing, the specimens were ground up with a pestle and mortar [18]. In a beaker, each crushed leaf sample was submerged in the necessary amount of water (1:1 w/v) and cooked for ten minutes at 100°C. After 24 hours in the lab at an average temperature of 28°C ± 2°C, crude extracts of the leaves of *N. lotus*, *P. scrobiculatum*, and *E. crassipes* were obtained. Cheesecloth and Whatman filter paper No. 42 (125 mm) were used to filter the infusions. The study's standard plant extract was created from the filtrate.

*Phytochemical Screening of Leaf Extracts of Hydrophytes*

Standard methods were used for quantitative phytochemical screening of the leaf extracts to determine the amounts of flavonoids, saponins, phenols, tannins, terpenoids, steroids, and cardiac glycosides [19].

*Flavonoids (mg/g)*

Using 100 mL of 80% aqueous methanol at room temperature, approximately 10 g of leaf samples from each hydrophyte (*N. lotus*, *P. scrobiculatum*, and *E. crassipes*) were extracted. A Whatman No. 42 filter paper was used to filter the solution into a 250 mL beaker that had already been weighed. After being moved into a water bath and allowed to evaporate until it was completely dry, the filtrate was weighed [20].

*Total Saponins (mg/g)*

A total of 100 mL of 20% aqueous ethanol was mixed with 20 g of each hydrophyte's powdered leaf sample. The mixture was heated to an estimated 55°C in a hot water bath for four hours while being continuously stirred. After filtering the mixture, another 200 mL of 20% ethanol was used to extract the residue. Over a water bath at roughly 90°C, the mixed extracts were reduced to 40 mL. After transferring the concentrate into a 250 mL separating funnel, 20 mL of diethyl ether was added, and the mixture was violently shaken. The ether layer was discarded and the aqueous layer was recovered. The purifying procedure was repeated. Next, n-butanol (60 mL) was added. Ten milliliters of 5% aqueous sodium chloride were used to wash the mixed n-butanol extracts twice. The saponin concentration was then measured after the residual solution was heated in a water bath for evaporation and further dried in an oven to achieve a consistent weight [20].

*Total Phenol (mg/g)*

Distilled water was used to assess the phenol content of hydrophyte samples; an estimated aliquot of samples was made up to approximately 1.5 mL. Following the addition of 10 mL of sodium carbonate, 0.5 mL of Folin-Ciocalteu reagent was applied. After that, the mixture was incubated at 37°C for one hour. At 750 nm, the absorbance values were measured.

Using the gallic acid calibration curve, the total phenolic content was calculated as (GAE)/g equivalent of extracts [20].

*Tannins (mg/g)*

A 100 mL volumetric flask was filled with 75 mL of water and 20 g of dried hydrophyte material. Approximately 5 mL of Folin-Ciocalteu reagent was added to 10 mL of $Na_2CO_3$ solution, and the mixture was shaken vigorously for about 30 minutes in the dark. The solution turned blue, and its absorbance was measured at 760 nm using a spectrophotometer. A standard curve was used to compare the tannic acid concentration, which was expressed as a percentage and in mg/g [20].

*Terpenoids (mg/g)*

After adding about 100 mg, or the initial weight-wi of dried plant extract, to 9 mL of ethanol for a full day, the mixture was filtered using Whatman filter paper. Ten millilitres of petroleum ether were used to extract the filtrate. The final weight-wf was recorded after the petroleum ether extract was isolated and completely dried in a glass vial that had been previously weighed. After the ether was fully evaporated, the yield (%) of total terpenoids was determined using the following formula [20].

$$\frac{W_i - W_f}{W_i} \times 100$$

*Steroid (mg/g)*

Two millilitres of chloroform and concentrated sulfuric acid ($H_2SO_4$) were carefully added to a test tube along with 1 mL of each plant extract. The absorbance at 420 nm was measured in relation to a cholesterol standard curve to determine the quantitative analysis. The presence of steroids was verified by the formation of a reddish-brown interface. Steroid values were reported in mg/g of extract using the SI unit [20].

*Cardiac Glycoside Content (mg/g)*

The Kellar-Killiani method was used to determine the cardiac glycoside content. Each plant extract was combined with one of the following quantities of glacial acetic acid (1 or 2 mL) and a trace amount of ferric chloride solution. To create a clear layer, 1 mL of strong sulfuric acid was carefully added along the test tube's sides. The presence of deoxy sugars, a characteristic of cardiac glycosides, is indicated by the creation of a brown ring at the contact interface. Digitoxin was used as the standard to obtain quantitative measurements of cardiac glycoside content at 530 nm by measuring absorbance. The results were represented in mg/g of extract as SI units [20].

*Data Analysis*

All experiments were conducted in triplicate and data collected were subjected to statistical analysis using SPSS to evaluate ANOVA, confidence intervals, significance testing, and LSD comparisons. Results were presented in the form of tables and charts for easy interpretation.

## Results And Discussion

### Fungal morphology

Table *1* presents the macromorphological and micromorphological characteristics of fungal samples identified in this study. Four fungal species were identified including *Aspergillus niger, Fusarium oxysporium,* and *Rhizopus nigricans. Aspergillus nige*r belongs to the family Asparagaceae and is characterized by velvety filamentous white growth that sporulates black powdery spores. It possesses a long septate hyphal growth with sporangiospore bearing brown spores and philaides at the apex when viewed under the microscope. *Fusarium oxysporium* belongs to the family Nectriaceae and is structurally identified as colonies that are typically white with pink or violet pigment. It possesses microscopic features such as hyaline, septate hyphae with distinct sickle-shaped multi-celled macroconidia with a foot cell at the base. *Mucor indicus* had yellowish-brown cottony colonies with branched, aseptate hyphae and spherical sporangia with

a unique microscopic feature of transparent hyaline structures. They are smooth-walled, usually branched and arising from hyphae. It is classified under the family Mucoraceae. *Rhizopus nigricans* belong to the family Mucoraeae and are recognized by its filamentous hyphae, which are coenocytic and mostly lacking septa.

The genus *Aspergillus* contains species that are etiologic agents of aspergillosis. They are the most ubiquitous in nature and are the major cause of pulmonary diseases. Examples include *A. flavus* and *A. niger*. Several species of Asparagaceae in the section Fumigati are morphologically the same [21]. *Fusarium oxysporum* species primarily cause Fusarium wilt [22], which globally impact plant yield resulting in significant agricultural loss . The soil-borne pathogen infects particularly the roots, parenchyma xylem vessels, and reduces water transportation, causing wilting, chlorosis, and necrosis. The disease cycle of *F. oxysporum* in the soil is dominated by long-lived chlamydospores with a dispersal pattern transported via microconidia within the vascular system [23]. Mucor species represent a significant group of invasive mycosis present in severely immunocompromised patients [24]. They are opportunistic pathogens, in which some species cause mucormycoses in humans and animals, while others hold significant economic value in food fermentation and bioengineering [25]. Rhizopus species are one of the main pathogens of postharvest storage equipment. The control methods of Rhizopus in previous studies suggests future directions of postharvest Rhizopus infection control using two aspects to analyze the pathogenic mechanism first in research and second in composite technology [26].

| S. No. | Macromorphology | Micromorphology | Suspected Organism | Family |
|---|---|---|---|---|
| 1 | Velvety filamentous white growth that sporulates black powdery spore | Long septate hyphal growth with sporangiospore bearing brown spores and philaid at its apex | *Aspergillus niger* van Tieghem (1867) | Aspergillaceae |
| 2 | Colonies are typically white with pink/violet pigment | Hyaline, septate hyphae with distinct sickle-shaped multi-celled macroconidia, with a foot cell at the base | *Fusarium oxysporium* Schltdl. (1824) | Nectriaceae |
| 3 | white to yellowish-brown cottony colonies with branched, aseptate hyphae and spherical sporangia | Hyaline (transparent) and smooth-walled, usually branched and arising from hyphae | *Mucor indicus* Lendn. (1908) | Mucoraceae |
| 4 | Filamentous, coenocytic. Usually lacking septa | Having long filamentous, and coenocytic (aseptate) | *Rhizopus nigricans* (Ehrenb.) Vuill (1902) | Rhizopodaceae |

**TABLE 1: Macro and micro-morphological characteristics of fungal species identified**

Figure *1A*-D presents four microphotographic images of fungal organisms identified in the present study. Figure *1A* presents *A. niger* which showcased long septate hyphal growth with sporangiospore bearing brown spores bearing philaides at the apex. Figure *1B* presents *M. indicus* showing smooth-walled hyaline branches arising from hyphae. Figure

*1C* presents *R. nigricans* which had long filamentous hyphae which are coenocytic. Figure *1D* presents *F. oxysporium* showing septate hyphae with distinct multi-celled macroconidia with a foot cell at the base.

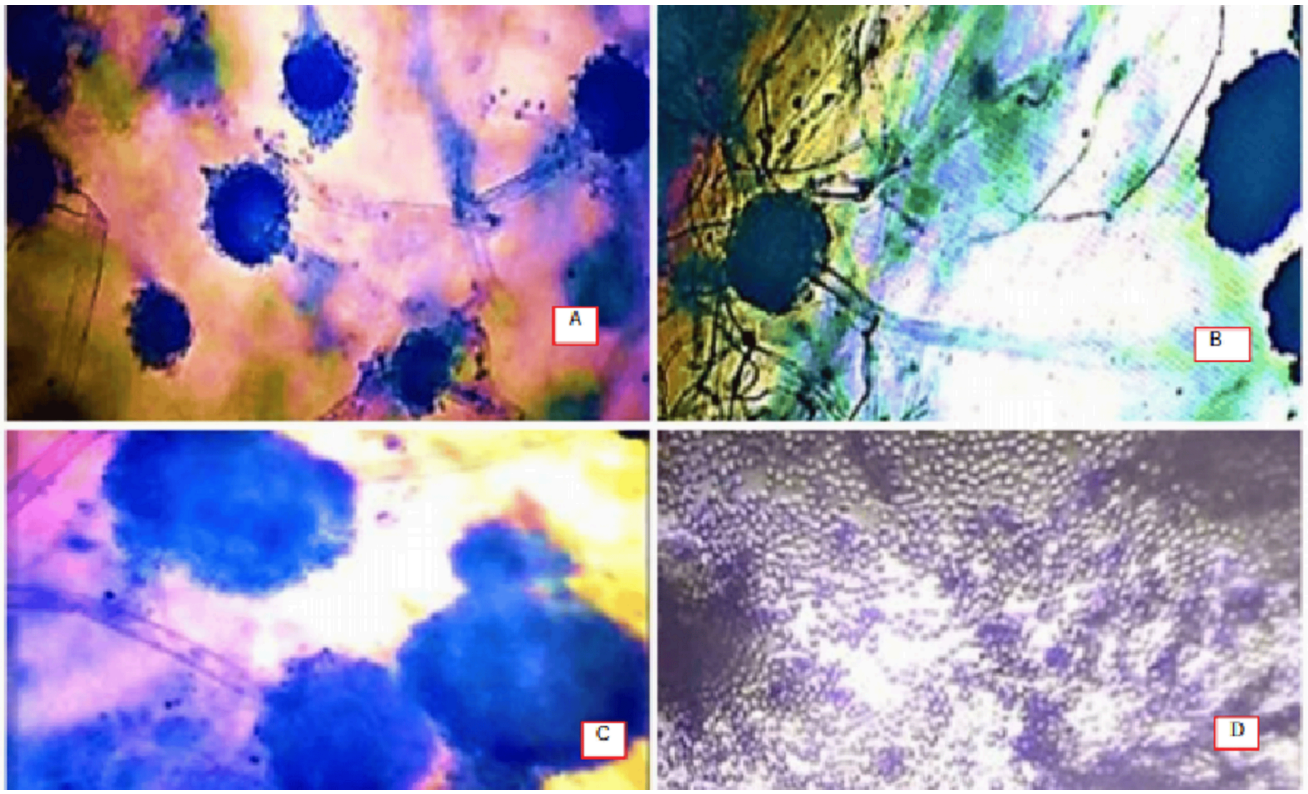



**FIGURE 1: Microphotographic images of the fungal species studied (A) Aspergillus niger with long, septate hyphae and brown sporangiospores; (B) Mucor indicus with smooth-walled, hyaline branches arising from hyphae; (C) Rhizopus nigricans with long, filamentous and coenocytic (aseptate) hyphae; (D) Fusarium oxysporium with distinct, multicellular macroconidia and a foot cell at the base**

### Fungal count of samples

Table *2* showcases the total fungal count (TFC) of 10 samples of *P. dactylifera* expressed in colony-forming units per gram ($\times 10^6$ cfu/g). The results revealed variations in fungal load among the samples, with mean counts ranging from $61.5 \times 10^6$ cfu/g in sample B to $235.5 \times 10^6$ cfu/g in sample D. The highest fungal count observed in sample D indicates a greater level of contamination, while the lowest count recorded in sample B suggests minimal fungal presence. Samples F ($201.5 \times 10^6$ cfu/g) and G ($183.5 \times 10^6$ cfu/g) also showed relatively high counts, whereas samples I ($66.0 \times 10^6$ cfu/g) and J ($90.0 \times 10^6$ cfu/g) recorded lower counts. Four fungal genera were isolated from samples A-J: *F. oxysporium*, *M. indicus*, *A. niger*, and *R. nigricans*. These isolates represents the dominant fungal contaminants in the analyzed samples.

The variations in fungal counts across the samples indicate the differences in fungal proliferation levels in fruits, possibly due to variations in sample conditions such as moisture content, exposure, or storage time. The mean fungal counts demonstrate that some samples contained higher microbial loads than others, reflecting differing levels of fungal activity.

The TFC results also revealed a significant variation in fungal contamination levels among the 10 (A-J) samples of *P. dactylifera* accessions, ranging from $61.5 \times 10^6$ cfu/g in sample B to $235.5 \times 10^6$ cfu/g in sample D. The highest fungal load observed in sample D indicates severe contamination, possibly linked to prolonged exposure to high moisture content, or poor storage conditions. Conversely, the lowest fungal count recorded in sample B suggests the presence of more effective storage or handling conditions that limited fungal growth. The detection of *F. oxysporium*, *M. indicus*, *A. niger*, and *R. nigricans* aligns with previous findings [27], which reportedly identified these studied species as the

dominant fungal contaminants in stored agricultural products such as groundnuts and maize due to their spore-forming ability and tolerance to fluctuating humidity [28]. Similarly, fungal species such as *A. niger* and *F. oxysporium* have been recorded as the most frequent post-harvest invaders capable of producing mycotoxins that pose public health risks [29]. The moderate to high fungal counts in this study indicate poor post-harvest hygiene and suboptimal storage conditions in date palm accessions, which are consistent with previous scientific observations which had emphasized that microbial contamination increased with temperature, relative humidity, and duration of storage [30]. Therefore, existing findings have reinforced the need for proper drying and airtight storage practices to prevent fungal proliferation and maintain product quality.

| S/N | Sample | TFC ($\times 10^6$ cfu/g) | Mean TFC ($\times 10^6$ cfu/g) |
|---|---|---|---|
| 1 | P312II (A) | 128,134 | 131.0 |
| 2 | P5R15 GRPII (B) | 64,59 | 61.5 |
| 3 | SUCHARY (C) | 136, 132 | 134.0 |
| 4 | DRY BROWN (D) | 264, 207 | 235.5 |
| 5 | TILGAR (E) | 177,129 | 153.0 |
| 6 | P17R14 GRP 11 (F) | 201,202 | 201.5 |
| 7 | P21R4 GRPII (G) | 184,183 | 183.5 |
| 8 | P21R15 GRP II (H) | 145,143 | 144.0 |
| 9 | P2R17 GRPIV (I) | 66,66 | 66.0 |
| 10 | GREEN TYPE (J) | 91,89 | 90.0 |

**TABLE 2: Fungi count of samples**

TFC, Total Fungal Count

### Zones of inhibition from culture plate assay

Table *3* presents the analysis of variance for zones of inhibition from the culture plate assay at a confidence interval of 95%. The results show the zones of inhibition in the studied fungal isolates, such as *F. oxysporium, R. nigricans, M. indicus*, and *A. niger*. Plant extracts determined the respective zones of inhibition measured. The mean values indicate varying degrees of antifungal effectiveness among the extracts. The significant value (alpha) was less than 0.05 at the 95% confidence level.

The extract of *N. lotus* showcased inhibition zones ranging from 9.50 mm to 15.50 mm, indicating moderate activity against the test fungi. *Paspalum scrobiculatum* exhibited lower inhibitory effects, with inhibition zones between 7.50 mm and 8.50 mm, with no observable inhibition against *A. niger*. Furthermore, *E. crassipes* demonstrated the highest antifungal activity among the plant extracts, with inhibition zones ranging from 15.00 mm to 18.50 mm across the test fungi. The control fluconazole had minimum inhibitory values ranging from 32 mm to 38 mm, confirming its strong antifungal potency. Overall, the inhibition zones varied among the extracts, indicating differences in antifungal efficacy and bioactive compound concentration.

The results of antifungal activities demonstrated distinct variations in the inhibitory effects of the hydrophyte extracts. *Eichhornia crassipes* showcased the highest antifungal activity with inhibition zones ranging from 15 mm to 19 mm, while *N. lotus* exhibited moderate inhibition (9.5-15.5 mm). *Paspalum scrobiculatum* had the lowest activity (7.5-8.5 mm) and no inhibition against *A. niger.* These results agree with previous findings [31], which emphasized that *E. crassipes* extract possesses potent antifungal properties against *C. albicans* and *A. niger*, but in this case no impact on *A. niger* was recorded thus, attributing the activity to the high phenolic and tannin contents. Similarly, in other investigations *N. lotus* extract exhibited moderate inhibition against *F. oxysporium* and *R. nigricans*, corroborating with the present study's findings [32]. The weak antifungal performance of *P. scrobiculatum* is consistent with previous findings, which had reported that limited antimicrobial potency of certain grass species was due to low concentrations of active phytochemicals [33]. The control drug, fluconazole, exhibited the largest inhibition zones (32-38 mm), which was expected, as synthetic antifungal agents are generally more concentrated and standardized in terms of bioactive components.

| Fungi | Source of Variation | Sum of Squares | df | Mean Square | F | Sig.* |
|---|---|---|---|---|---|---|
| *Fusarium* | Between groups | 652.38 | 3 | 217.46 | 9.56 | >.001 |
| | Within groups | 9.5 | 4 | 2.38 | | |
| | Total | 661.88 | 7 | | | |
| *Rhizopus* | Between groups | 910.5 | 3 | 303.5 | 34.69 | >.001 |
| | Within groups | 35 | 4 | 8.75 | | |
| | Total | 945.5 | 7 | | | |
| *Mucor* | Between groups | 706 | 3 | 235.33 | 470.67 | >.001 |
| | Within groups | 2 | 4 | 0.5 | | |
| | Total | 708 | 7 | | | |
| *Aspergillus* | Between groups | 1434.5 | 3 | 478.17 | 1912.67 | >.001 |
| | Within groups | 1 | 4 | 0.25 | | |
| | Total | 1435.5 | 7 | | | |

**TABLE 3: Analysis of variance for zones of inhibition from culture plate assay**

### Minimum inhibitory concentration of hydrophytes

Table *4* presents the minimum inhibitory concentration (MIC) values of the hydrophyte extracts against four fungal isolates *F. oxysporium*, *R. nigricans*, *M. indicus*, and *A. niger* expressed in mg/mL. The MIC represents the lowest concentration of extract required to inhibit visible fungal growth. The extract of *N. lotus* exhibited MIC values ranging from 12.50 mg/mL to 50.00 mg/mL, showing the lowest inhibitory concentration against *R. nigricans* (12.50 mg/mL) and

the highest against *A. niger* (50.00 mg/mL). *Paspalum scrobiculatum* showed a uniform MIC value of 50.00 mg/mL against *F. oxysporium*, *R. nigricans*, and *M. indicus*, with no inhibition observed against *A. niger*. *Eichhornia crassipes* demonstrated the lowest MIC values among the tested extracts, ranging from 12.50 mg/mL to 25.00 mg/mL, indicating greater inhibitory effectiveness across all fungi. Overall, the variation in MIC values among the extracts indicates differences in their antifungal potency and the concentration required to suppress fungal growth.

The MIC results further confirmed the antifungal strength of the extracts. *Eichhornia crassipes* displayed the lowest MIC values (12.50-25.00 mg/mL), indicating high potency, while *N. lotus* showed moderate MIC values (12.50-50.00 mg/mL). *Paspalum scrobiculatum* exhibited the highest MIC (50.00 mg/mL), reflecting weak inhibitory capacity. This result corresponds with previous findings that *E. crassipes* extracts inhibited *F. oxysporium* and *A. niger* at low MIC values due to the presence of phenolics and alkaloids [34]. Similarly, *N. lotus* required higher concentrations to achieve similar inhibition, a finding that mirrors the moderate MIC observed in the current study [35]. The limited activity of *P. scrobiculatum* aligns with past findings which attributed high MIC values to the low concentration of bioactive compounds and possible thermal degradation during extraction [36]. Hence, the varying MIC values in this study further illustrate that the antifungal potency of plant extracts depends not only on the presence of phytochemicals but also on their bioavailability and synergistic interactions.

| Extract | *Fusarium oxysporium* | *Rhizopus nigricans* | *Mucor indicus* | *Aspergillus niger* |
|---|---|---|---|---|
| *Nymphaea lotus* | 25.00 | 12.50 | 25.00 | 50.00 |
| *Paspalum scrobiculatum* | 50.00 | 50.00 | 50.00 | – |
| *Eichhornia crassipes* | 12.50 | 12.50 | 12.50 | 25.00 |

**TABLE 4: Minimum inhibitory concentration of studied hydrophytes**

### Phytochemical content of extracts of hydrophytes

Table *5* presents the quantitative phytochemical composition of the hydrophyte extracts, showing the concentrations (mg/g) of six bioactive compounds namely; tannins, cardiac glycoside, terpenoids, steroids, phenols, and flavonoids. The results indicate that the three extracts contained all the tested phytochemicals, though in varying concentrations.

The extract of *N. lotus* contained moderate levels of tannins (1.89 mg/g), cardiac glycosides (2.64 mg/g), and terpenoids (2.26 mg/g), with moderate levels of steroids (1.50 mg/g), phenols (1.49 mg/g), and flavonoids, the lowest of which was (0.10 mg/g). *Paspalum scrobiculatum* exhibited the highest concentration of cardiac glycosides (2.28 mg/g) but relatively low levels of tannins (0.25 mg/g) and phenols (0.25 mg/g). In contrast, *Eichhornia crassipes* recorded the highest contents of tannins (2.65 mg/g), steroids (2.75 mg/g), and phenols (2.30 mg/g), with moderate levels of terpenoids (1.68 mg/g), cardiac glycosides (0.50 mg/g) and flavonoids (0.43 mg/g).

Phytochemical screening revealed the presence of key secondary metabolites in all extracts, all in varying concentrations. *Eichhornia crassipes* had the highest levels of tannins (2.65 mg/g), steroids (2.75 mg/g), and phenols (2.30 mg/g), followed by *N. lotus*, while *P. scrobiculatum* recorded the lowest overall levels of all studied bioactive compounds. These results are in line with recent findings on *E. crassipes* [37,38]. This species contains significant quantities of phenolic compounds and steroids, which contribute to the majority of antimicrobial and antioxidant activities. Likewise, moderate concentrations of glycosides and terpenoids were reported in *Nymphaea lotus*, supporting its observed intermediate antifungal potency [39]. The relatively low quantity of phenolic and tannin content in *P. scrobiculatum* agrees with recent

investigations that grasses often possess lower levels of bioactive metabolites compared with broad-leaved aquatic plants [39]. Since phenols and tannins are known to denature microbial enzymes and disrupt cell walls, their high levels in *E. crassipes* could explain its superior antifungal performance observed in this study.

| Extract | Tannin (mg/g) | Cardiac glycoside (mg/g) | Terpenoid (mg/g) | Steroid (mg/g) | Phenol (mg/g) | Flavonoid (mg/g) |
|---|---|---|---|---|---|---|
| *Nymphaea lotus* | 1.89±0.00 | 2.64±0.03 | 2.26±0.04 | 1.50±0.02 | 1.49±0.00 | 0.10±0.00 |
| *Paspalum scrobiculatum* | 0.25±0.00 | 2.27±0.01 | 2.21±0.06 | 1.64±0.45 | 0.25±0.01 | 0.48±0.01 |
| *Eichhornia crassipes* | 2.65±0.05 | 0.50±0.00 | 1.68±0.06 | 2.75±0.12 | 2.30±0.01 | 0.43±0.00 |

**TABLE 5: Phytochemical content of the extract of hydrophytes studied**

Mean ± standard deviation

## Conclusions

The findings in the present study revealed that *E. crassipes* exhibited the strongest antifungal activity, evidenced by the largest zones of inhibition and lowest MIC, indicating its high potency against fungal pathogens such as *F. oxysporium, R. nigricans, M. indicus,* and *A. niger*. *Nymphaea lotus* demonstrated moderate antifungal effects, while *P. scrobiculatum* displayed the least inhibitory activity. Phytochemical analysis showed that the extracts contained important bioactive compounds, including tannins, phenols, steroids, terpenoids, cardiac glycosides, and flavonoids, with *E. crassipes* recording the highest concentrations of these metabolites. The observed antifungal activity corresponds with the phytochemical composition, as higher levels of phenols and tannins correlated with stronger inhibition of fungal growth. Hydrophytes, particularly *E. crassipes* and *N. lotus*, possess significant antifungal properties that can be attributed to their rich phytochemical content. These plants represent a potential source of natural antifungal agents that could serve as alternatives or supplements to synthetic fungicides in managing fungal infections in agricultural and clinical settings. Based on the findings in the present study, The antifungal extracts of *E. crassipes* and *N. lotus* should be explored as eco-friendly bio fungicides to manage post-harvest fungal contamination in stored agricultural products, especially in humid tropical regions.

## Appendices

| Descriptives | Group/ Model | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum | Between-Component Variance |
|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | Lower Bound | Upper Bound | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Fusarium* | WL | 2 | 13.5 | 2.12132 | 1.5 | -5.5593 | 32.5593 | 12 | 15 | |
| | PG | 2 | 7.5 | 0.70711 | 0.5 | 1.1469 | 13.8531 | 7 | 8 | |
| | CS | 2 | 17.5 | 2.12132 | 1.5 | -1.5593 | 36.5593 | 16 | 19 | |
| | FL | 2 | 32 | 0 | 0 | 32 | 32 | 32 | 32 | |
| | Total | 8 | 17.625 | 9.72387 | 3.43791 | 9.4956 | 25.7544 | 7 | 32 | |
| | Model—Fixed Effects | | | | 1.5411 | 0.54486 | 16.1122 | 19.1378 | | |
| | Random Effects | | | | 5.21366 | 1.0328 | 34.2172 | | | 107.542 |
| *Rhizopus* | WL | 2 | 15.5 | 0.70711 | 0.5 | 9.1469 | 21.8531 | 15 | 16 | |
| | PG | 2 | 8.5 | 0.70711 | 0.5 | 2.1469 | 14.8531 | 8 | 9 | |
| | CS | 2 | 16 | 5.65685 | 4 | -34.825 | 66.8248 | 12 | 20 | |
| | FL | 2 | 37 | 1.41421 | 1 | 24.2938 | 49.7062 | 36 | 38 | |
| | Total | 8 | 19.25 | 11.622 | 4.10901 | 9.5337 | 28.9663 | 8 | 38 | |
| | Model—Fixed Effects | | | | 2.95804 | 1.04583 | 16.3463 | 22.1537 | | |
| | Random Effects | | | | 6.15934 | -0.3518 | 38.8518 | | | 147.375 |
| *Mucor* | WL | 2 | 12.5 | 0.70711 | 0.5 | 6.1469 | 18.8531 | 12 | 13 | |
| | PG | 2 | 7.5 | 0.70711 | 0.5 | 1.1469 | 13.8531 | 7 | 8 | |
| | CS | 2 | 15.5 | 0.70711 | 0.5 | 9.1469 | 21.8531 | 15 | 16 | |
| | FL | 2 | 32.5 | 0.70711 | 0.5 | 26.1469 | 38.8531 | 32 | 33 | |
| | Total | 8 | 17 | 10.057 | 3.55568 | 8.5922 | 25.4078 | 7 | 33 | |
| | Model—Fixed Effects | | | | 0.70711 | 0.25 | 16.3059 | 17.6941 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Random Effects | | | | 5.42371 | -0.2607 | 34.2607 | | | 117.417 |
| *Aspergillus* | WL | 2 | 9.5 | 0.70711 | 0.5 | 3.1469 | 15.8531 | 9 | 10 | |
| | PG | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | CS | 2 | 15 | 0 | 0 | 15 | 15 | 15 | 15 | |
| | FL | 2 | 36.5 | 0.70711 | 0.5 | 30.1469 | 42.8531 | 36 | 37 | |
| | Total | 8 | 15.25 | 14.3203 | 5.063 | 3.2779 | 27.2221 | 0 | 37 | |
| | Model—Fixed Effects | | | | 0.5 | 0.17678 | 14.7592 | 15.7408 | | |
| | Random Effects | | | | 7.73116 | -9.354 | 39.854 | | | 238.958 |

**TABLE 6: Descriptive statistics for zones of inhibition**

WL, Water lily (*Nymphaea lotus*); PG, *Paspalum scrobiculatum*; CS, *Eichhornia crassipes*; FL, Fluconazole

| **Dependent Variable** | **(I) Extract** | **(J) Extract** | **Mean Difference (I-J)** | **Std. Error** | **Sig.** | **95% Confidence Interval** | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Fusarium* | *LSD* | WL | PG | 6.00000* | 1.5411 | 0.018 | 1.7212 | 10.2788 |
| | | | CS | -4 | 1.5411 | 0.06 | -8.2788 | 0.2788 |
| | | | FL | -18.50000* | 1.5411 | < .001 | -22.7788 | -14.2212 |
| | | PG | WL | -6.00000* | 1.5411 | 0.018 | -10.2788 | -1.7212 |
| | | | CS | -10.00000* | 1.5411 | 0.003 | -14.2788 | -5.7212 |
| | | | FL | -24.50000* | 1.5411 | < .001 | -28.7788 | -20.2212 |
| | | CS | WL | 4 | 1.5411 | 0.06 | -0.2788 | 8.2788 |
| | | | PG | 10.00000* | 1.5411 | 0.003 | 5.7212 | 14.2788 |
| | | | FL | -14.50000* | 1.5411 | < .001 | -18.7788 | -10.2212 |
| | | FL | WL | 18.50000* | 1.5411 | < .001 | 14.2212 | 22.7788 |
| | | | PG | 24.50000* | 1.5411 | < .001 | 20.2212 | 28.7788 |
| | | | CS | 14.50000* | 1.5411 | < .001 | 10.2212 | 18.7788 |
| Rhizopus | LSD | WL | PG | 7 | 2.95804 | 0.077 | -1.2128 | 15.2128 |
| | | | CS | -0.5 | 2.95804 | 0.874 | -8.7128 | 7.7128 |
| | | | FL | -21.50000* | 2.95804 | 0.002 | -29.7128 | -13.2872 |
| | | PG | WL | -7 | 2.95804 | 0.077 | -15.2128 | 1.2128 |
| | | | CS | -7.5 | 2.95804 | 0.064 | -15.7128 | 0.7128 |
| | | | FL | -28.50000* | 2.95804 | < .001 | -36.7128 | -20.2872 |
| | | CS | WL | 0.5 | 2.95804 | 0.874 | -7.7128 | 8.7128 |
| | | | PG | 7.5 | 2.95804 | 0.064 | -0.7128 | 15.7128 |
| | | | FL | -21.00000* | 2.95804 | 0.002 | -29.2128 | -12.7872 |
| | | FL | WL | 21.50000* | 2.95804 | 0.002 | 13.2872 | 29.7128 |
| | | | PG | 28.50000* | 2.95804 | < .001 | 20.2872 | 36.7128 |
| | | | CS | 21.00000* | 2.95804 | 0.002 | 12.7872 | 29.2128 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mucor | LSD | WL | PG | 5.00000* | 0.70711 | 0.002 | 3.0368 | 6.9632 |
| | | | CS | -3.00000* | 0.70711 | 0.013 | -4.9632 | -1.0368 |
| | | | FL | -20.00000* | 0.70711 | < .001 | -21.9632 | -18.0368 |
| | | PG | WL | -5.00000* | 0.70711 | 0.002 | -6.9632 | -3.0368 |
| | | | CS | -8.00000* | 0.70711 | < .001 | -9.9632 | -6.0368 |
| | | | FL | -25.00000* | 0.70711 | < .001 | -26.9632 | -23.0368 |
| | | CS | WL | 3.00000* | 0.70711 | 0.013 | 1.0368 | 4.9632 |
| | | | PG | 8.00000* | 0.70711 | < .001 | 6.0368 | 9.9632 |
| | | | FL | -17.00000* | 0.70711 | < .001 | -18.9632 | -15.0368 |
| | | FL | WL | 20.00000* | 0.70711 | < .001 | 18.0368 | 21.9632 |
| | | | PG | 25.00000* | 0.70711 | < .001 | 23.0368 | 26.9632 |
| | | | CS | 17.00000* | 0.70711 | < .001 | 15.0368 | 18.9632 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Aspergillus | LSD | WL | PG | 9.50000* | 0.5 | < .001 | 8.1118 | 10.8882 |
| | | | CS | -5.50000* | 0.5 | < .001 | -6.8882 | -4.1118 |
| | | | FL | -27.00000* | 0.5 | < .001 | -28.3882 | -25.6118 |
| | | PG | WL | -9.50000* | 0.5 | < .001 | -10.8882 | -8.1118 |
| | | | CS | -15.00000* | 0.5 | < .001 | -16.3882 | -13.6118 |
| | | | FL | -36.50000* | 0.5 | < .001 | -37.8882 | -35.1118 |
| | | CS | WL | 5.50000* | 0.5 | < .001 | 4.1118 | 6.8882 |
| | | | PG | 15.00000* | 0.5 | < .001 | 13.6118 | 16.3882 |
| | | | FL | -21.50000* | 0.5 | < .001 | -22.8882 | -20.1118 |
| | | FL | WL | 27.00000* | 0.5 | < .001 | 25.6118 | 28.3882 |
| | | | PG | 36.50000* | 0.5 | < .001 | 35.1118 | 37.8882 |
| | | | CS | 21.50000* | 0.5 | < .001 | 20.1118 | 22.8882 |

**TABLE 7: Post hoc tests**

*The mean difference is significant at the 0.05 level. WL, Water lily (Nymphaea lotus); PG, Paspalum scrobiculatum; CS, Eichhornia crassipes; FL, Fluconazole

## Additional Information

### Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

**Concept and design:** Onyinyechi P. Okpara, Emmanuel J. Ode

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## Disclosures

**Plant subjects:** The study involves use of specimen, the voucher no FUAMHS56738-1-3, FUAMHS59938-1-10, was submitted to Department of Plant Science and Biotechnology herbarium.

**Human subjects:** All authors have confirmed that this study did not involve human participants or tissue.

**Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue.

**Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following:

- **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work.
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

### Data Availability Statements

All data generated or analyzed during this study are included in this published article and/or its appendices.

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