In Vitro Antifungal Activity of *Bauhinia monandra* (Kurz) Leaf Extracts Against Fungal Pathogens Isolated from Spoilt *Musa paradisiaca* L.

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1 Introduction

Foods are organic compounds sourced from plants and animals ingested to provide moisture, protein, fat, carbohydrate, minerals and other organic compounds. Plantains are important food crops that serve as staple foods for human consumption as well as a source of income generation for the economically growing countries. *Musa paradisiaca*, the cooking banana and sometimes referred to as plantain, is a member of the Musaceae family with origin from Southeast Asia (Nwaiwu et al., 2012). It is also cultivated across tropic and subtropical climates of the globe (Vu et al., 2018). It is the fourth most significant food crop in the world with Cameroon as the world leading producer that exports approximately 4.31 million tons yearly (FAO, 2019). *Musa paradisiaca* contains diverse important nutritional and economic values. Compared to other fruits and vegetables, plantains have greater total dietary fibre content, particularly in hemicelluloses (Imam & Aktar, 2011). According to Sojinu et al. (2021), plantains, both ripe and unripe, are beneficial in the treatment of some ailments and diseases (such as goitre) due to their high concentration of essential nutrients, antioxidants and other biologically active components. Economically, different parts are utilized as food, fodder and compost (Agama-Acevado et al., 2016), biorefinery (Martinez-Ruano et al., 2018), food packaging and production of acids.

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Plantains are susceptible to pests and diseases which are major constraints in production. Different microbial strains are found to infest and deteriorate foods including plantain due to the ability to invade and infect plants pre and postharvest. Fungi are the most crucial and common pathogens responsible for crop diseases. These fungi infect a wide range of fruits and vegetables during storage and transportation (Abdullah et al., 2016). Fungal invasion in plantain can be due to mycotoxins or the extracellular enzymes that aid in spoilage. Studies have shown that in fruits and herbs, an extensive range of different fungal species are responsible for characteristic problems that include features, nutritional merit, organoleptic traits and deficient shelf life with some cases of allergic or toxic disorders among consumers caused indirectly by fungi from the production of allergens or mycotoxins. Some phytopathogenic fungi associated with the deterioration of plantain include *Fusarium* sp., *Aspergillus flavus, Aspergillus niger, Colletotrichum* sp. and *Penicillium* sp. (Umeh et al., 2017; Ejimofor et al., 2022; Hassen et al., 2022).

Synthetic fungicides have become ingrained throughout agriculture. Over 110 novel fungicides have been produced since the discovery of the first synthetic fungicide, phenylmercury acetate, in 1913, thus reducing loss in production yield. In spite of their benefits, the extensive use of fungicides has posed some risks to the environment and human health because of their toxicity and long-lasting impacts. It has also amounted to the development of resistant strains (Ons et al., 2020). Plants are the richest, most cost-effective and guaranteed alternative source of antimicrobials and contain different phytochemicals. These ensure plants subsist as aetiologies of pharmaceutics used in traditional medicine and medicinal preparations (Ncube et al., 2018). Extensive studies on many medicinal plants are in view with a focus on discovering more potent and less toxic compounds from them. *Bauhinia monandra* is a tropical plant greatly employed in traditional medicine particularly in the management of diabetes. Additionally, the seed extracts have been shown to have hypoglycaemic, antioxidant, antimicrobial, anti-inflammatory, anti-nociceptive and antimicrobial properties (Aderogba et al., 2006; Ajiboye et al., 2015; Solomon et al., 2016). With antimalarial, antiviral, antibacterial, antifungal, antidiarrheal and antispasmodic properties that have been studied, several *Bauhinia* species are also used as traditional medicines around the world (Onyije et al., 2012). The leaves of *B. purpurea* Linn, *B. forficata* Link and *B. monandra* Kurz are extensively used to cure diabetes in Brazil. Despite many reports about the pharmacological properties of its different species, only few reports have been published regarding its antimicrobial activity. There is a paucity of information in the research about the antifungal activity of *B. monandra*. Hence, this study was conducted to evaluate the in vitro antifungal activity of *B. monandra* leaf extracts against some phytopathogenic fungi.

2 Materials and Methods

2.1 Fungal Isolates and Inoculum Quantification

A total of 24 healthy and randomly spoilt plantain fingerlings that were obtained from Agbara, Iju and Ota markets (being major collection and retailing points) in Nigeria were labelled accordingly. These samples were immediately conveyed in Ziploc bags to the microbiology laboratory for analysis. The plantain fingerlings were subjected to a 2-minute surface sterilization in 1% hypochlorite and three thorough rinses in sterile distilled water. The samples were then aseptically placed on sterile filter papers to drain excess moisture. A sterile scalpel was used to cut out four pieces from the margin of the lesion, and the mycoflora from the plantain samples were isolated using PDA supplemented with 1 mL of 10% chloramphenicol after incubation at 25 °C for 5–7 days (Jha, 1995). The fungal isolates were phenotypically characterized by microscopy based on the observations for hyphal type, septation of hyphae (wall type of hyphae), spore colour, shape and arrangement and rhizoids or foot cells as described in Barnett and Hunter's atlas of mycology and compendium of fungus (1987).

2.2 Plant Collection and Identification

Leaves of *Bauhinia monandra* were harvested for use from the Covenant University Ota, Ogun State, Nigeria, between February and April 2022 in a sterile polythene bag and transported to the biology laboratory for identification by a botanist in the Biological Sciences Department. Authentication of the already identified plant species (voucher specimen number: Bm/Bio/H822) was conducted at the Herbarium Section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, and issued a forestry herbarium identification number of B. monandra-FHI No: 112777.

2.3 Preparation of Aqueous and Ethanolic Extracts

The mature disease-free leaves were rinsed to eliminate dust and other foreign particles and air-dried under a shade at ambient temperature for 3 weeks (Vinoth et al., 2011). Dried leaves were ground into powder using a sterile blender and preserved in airtight bottles at room temperature (25–30 °C) until use. The powdered leaves were extracted in aqueous and ethanol solvents by soaking 200 g of the leaves in 1000 mL of each of the aqueous and ethanol solutions to macerate for 2–3 days while stirring frequently to ensure that all soluble materials were dissolved. Following maceration, the combinations were filtered using Whatman's No. 1 filter paper and muslin cloth. A rotary evaporator was used to concentrate the filtrates that

were produced, and they were then stored at 4 °C for later use. After the solvents were recovered under pressure, slurried extracts were obtained (Oniha et al., 2021). The crude extracts obtained were then kept at 4 °C for subsequent use. The concentrated extracts were later dissolved in appropriate volumes of dimethyl sulfoxide (DMSO) to make varying crude concentrations (1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL where 1000 mg/mL was used for the antifungal assay) for antifungal assessment. All the stock solutions were stored in sterile capped bottles, labelled accordingly and stored at 4 °C for analysis. Each antifungal test was carried out in three replicates against each fungal isolate.

2.4 Phytochemical Screening of Plant Extracts

Qualitative chemical tests were conducted to check for the presence of anthraquinones, tannins, saponins, steroids, cardiac glycosides, flavonoids, terpenoids and alkaloids according to the method of Sofowora (1993).

2.5 In Vitro Antifungal Activity of Crude Extracts of **Bauhinia monandra**

A loopful of the fresh subcultures of each of the fungal isolates was picked from the Petri plate using a sterile inoculating loop and transferred into sterile McCartney bottles containing 1 mL of distilled water to match 0.5 McFarland standards. Antifungal activity of the crude extracts was conducted using the agar-well diffusion method as illustrated by Cheesbrough (2006) after the potato dextrose agar medium (Oxoid) was prepared according to the manufacturer's instructions. After this, the standard dose was prepared by dissolving 1000 mg of crude extract in 1 mL of DMSO (1:1) for both the aqueous and ethanolic extracts (stock concentration for the antifungal test). Ketoconazole (100 mg/mL), the antifungal agent, was used as the positive control, while DMSO served as the negative control. The standardized test organisms were used to seed the surfaces of the agar plates. Using cork borers, wells were made in the agar and 0.2 mL of each concentration of the plant extract was introduced. The plates were incubated after being allowed for an hour to allow the extract to fully diffuse into the agar pores. Over the course of 3–5 days of incubation at room temperature, the plates were examined for zones of inhibition, which were quantified and reported accordingly. The negative and positive control plates contained no extracts but ketoconazole (positive) and DMSO (negative), respectively.

All the sensitivity tests were carried out in duplicates and the mean zones of inhibition were recorded appropriately. MFC was defined as the lowest extract concentration that showed no visible growth after incubation time, and MIC was the lowest concentration showing minimal growth. The diameters measured from the zones of inhibition of the in vitro antifungal activity were analysed as the average of two replicates.

2.6 Data Analysis

Significant differences between and within the averages of treatments and controls were analysed using ANOVA at $p \leq 0.05$ and post hoc tests. Statistical analyses were computed using the SPSS version 20 software package.

3 Results and Discussion

Spoilage of foods results from diverse microbiological, physical or chemical activities, and these mechanisms are not always mutually exclusive since rotting caused by one process might lead to another (Sahu & Bala, 2017). Diverse microorganisms can be found to infest and contaminate perishable foods including a plantain (Sahu & Bala, 2017). There is a high possibility that these microorganisms came in contact with plantain during cultivation, harvesting, storage and transportation to the market, thereby infecting the plant at both pre- and post-harvest stages. These microorganisms are associated with soil, air and contaminated environments. The contamination of plantain by fungi generally affects its economic value. Consequently, these contaminated plantains can affect the health of the general consumers. Additionally, diverse phytopathogenic fungi that infect plantains and oranges vary depending on the species and environmental factors. From this study, the results of the isolation and characterization of mycofloral pathogens based on their cultural and microscopic features from the contaminated plantain samples are shown in Table 1 and include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Geotrichum* sp., *Rhizopus* sp. and *Alternaria* sp. The results of this study corroborate the findings of Abdullah et al. (2016), Ejimofor et al. (2022) and Sani

Table 1 Preliminary identification of fungal pathogens

Code	Cultural features	Microscopic features	Preliminary identification
P1, 2 and 3	Black cottony growth with yellow colour on the reverse of the plate	Radiate conidial heads, septate hyphal strands with black spores suspended in a conidial sac attached to conidiophores with the spores arranged in chains	*Aspergillus niger*
P1, 2 and 3	Lemon green powdery growth with yellow colour on the reverse of the plate	Hyaline septate hyphae with bluish-green spores suspended in a conidial sac	*Aspergillus flavus*
P1, 2 and 3	Brown powdery growth with yellow colour on the reverse of the plate	Brown spores contained in a conidial sac arranged from inside out in chains and suspended in a hyaline conidiophore	*Aspergillus fumigatus*
P1 and 2	Grey mycelial strands with brown tips	Thick-walled non-septate hyphal strands bearing brown oval-shaped spores contained in a sporangium	*Rhizopus*

Key: P1 represents Agbara market, (P2) Iju market, and (P3) Oja-Ota market

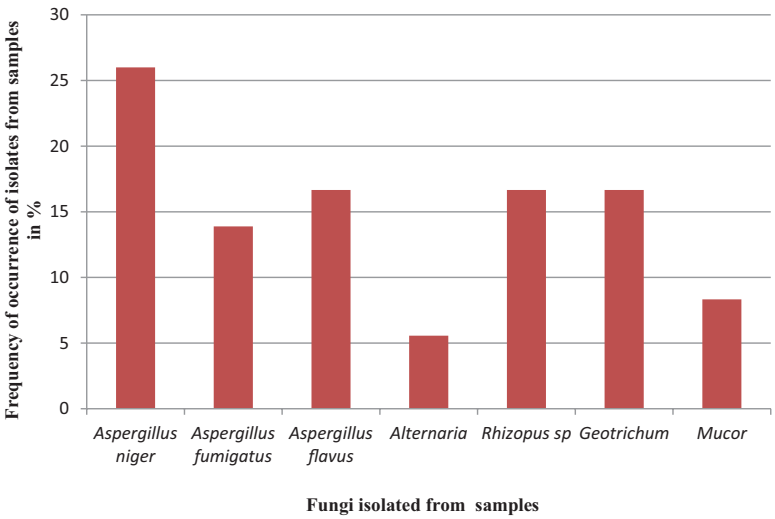

Fig. 1 Frequency of occurrence of fungal isolates from *Musa paradisiaca*

Table 2 Phytochemical screening of *B. monandra* leaf extracts

Tests	Aqueous	Ethanol
Carbohydrates	+	–
Tanins	+	–
Saponins	+	–
Glycosides	–	–
Alkaloids	–	–
Phenols	–	–
Terpenoids	–	+
Cardiac glycosides	+	+
Flavonoids	+	–
Coumarins	–	+
Steroids	–	+
Anthocyanins	–	–
Acids	–	–

Key: (+), present and (–), absent

and Kasim (2019) that published similar strains of phytopathogenic fungi in their studies. The frequency of fungi isolated from the plantain samples is presented in Fig. 1.

Results of qualitative phytochemical screening showed the presence of carbohydrates, cardiac glycosides, phenols, tannins, saponins, anthocyanin, betacyanin, coumarins and terpenoids in the leaf extracts. Carbohydrates, tannins, saponins, flavonoids, cardiac glycosides and flavonoids were present in the aqueous extract only, while terpenoids, cardiac glycosides, coumarins and steroids were present in the ethanolic extract only (Table 2). Alkaloids, phenol, anthocyanin and acid were

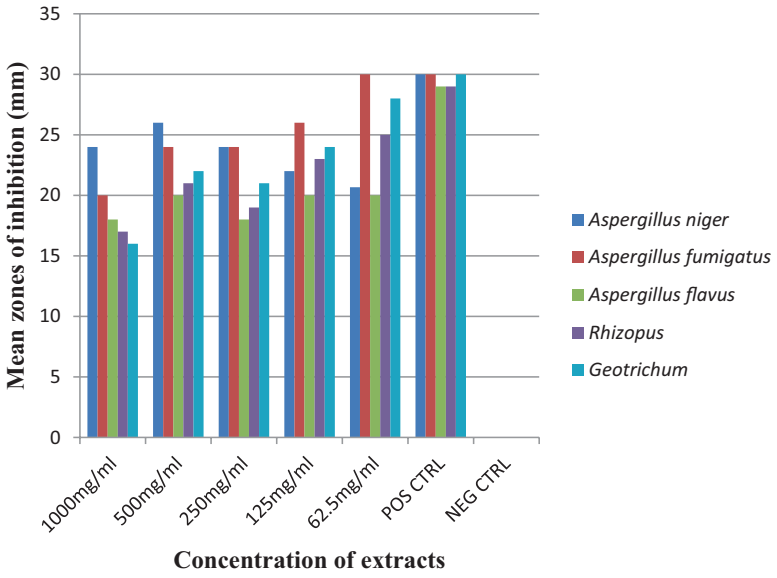

Fig. 2 Mean zones of inhibition for antifungal activity of aqueous leaf extract of *B. monandra* against fungal isolates

absent in both extracts. Our findings in this study support the reports of Ajiboye et al. (2015) that revealed the presence of flavonoids, tannins, steroids, terpenoids, saponins, cardiac glycosides and phenols in the leaf extract. Gizaw et al. (2022) stated that phytochemicals are responsible for the antifungal activity exhibited by plant extracts.

The results of the antifungal assessment of the aqueous and ethanolic extracts at different concentrations are presented in Figs. 2 and 3. Our findings revealed that both extracts had varying inhibitory activities against the fungal isolates at different concentrations.

Secondary metabolites from medicinal plants have antimicrobial properties in which their active ingredients negatively affect the development and metabolism of microorganisms. Their screening can offer a substitute for creating chemical fungicides that are reasonably safe and economical (Nxumalo et al., 2021). The observed antifungal activity of the leaf extracts was dependent on both the concentration and polarity of the extraction solvent used, thus reducing concentrations with a corresponding reduction in the zones of inhibition. However, there is a paucity of information on the antifungal activity of *B. monandra* against some fungal species isolated in this study compared to other species of the genus which include *B. variegate* and *B. racemosa* with similarities in morphology and phytochemicals.

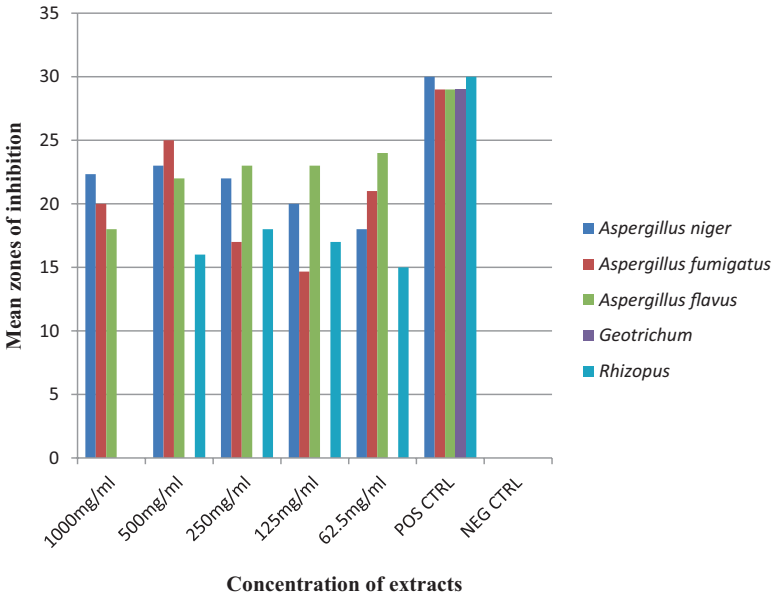

Fig. 3 Mean zones of inhibition for antifungal activity of ethanol leaf extract of *Bauhinia monandra* against fungal isolates

4 Conclusion

The findings of this study emphasized the need for preventing both pre- and post-harvest fungal diseases, with a focus on the high rate of spoiling in plantain production. Varying degrees of antifungal activity were observed in both extract types of *B. monandra* tested against the fungal pathogens depending on the extract type and concentrations. Thus, extracts of medicinal plants possess antifungal potentials that will aid in the prevention of fungal infections in plantains, thereby greatly minimizing spoilage and fungal infection. Further studies need to be conducted on extracts of *B. monandra* to fully uncover its antimicrobial properties as well as focus on the development of novel antifungal plant extracts as pre- and post-harvest antifungal agents.

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