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Research Article

ANTIFUNGAL ACTIVITY OF *MANGIFERA INDICA* LEAF EXTRACT ON FUNGI ISOLATED FROM BREAD VENDED WITHIN BAKORI, NIGERIA

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ABSTRACT

Objective: The present study was carried out to determine the antifungal activity of *Mangifera indica* leaves extract on fungi isolated from bread vended within Bakori.Fungi are the main infectious agents in plants and were reported to cause alterations during developmental stages including post-harvest

Methods: The powdered form of *M. indica* leaves was used to prepare the extract using ethanol, the leaves were air dried at room temperature for 10 days.

Results: The chemical test was carried out to identify the secondary metabolites, some of the metabolites that were present include alkaloids, saponins, flavonoids, steroids, and tannins. Both fungal isolates were identified on the basis of morphological characteristics as *Aspergillus niger, Aspergillus flavus*, and *Mucor* spp. The antifungal activity of *M. indica* leaves extract was determined using agar well diffusion method on *Aspergillus* and *Mucor* spp. The results showed that the extract was found to inhibit *A. niger* at 100 mg/ml, 50 mg/ml, and 25 mg/ml with 13.9 mm, 11.5 mm, and 8.0 mm, respectively, and *A. flavus* at 100 mg/ml, 50 mg/ml, and 25 mg/ml with 13.6 mm, 11.2 mm, and 8.1 mm, respectively, while *Mucor* spp. was found to be resistant at 25 mg/ml while 100 mg/ml and 50 mg/ml showed an activity. Minimum inhibitory concentration result showed a promising activity against *Aspergillus* spp. at 25 mg/ml while *Mucor* spp. at 50 mg/ml.

Conclusion: Therefore, *M. indica* leaf extracts can be used in the treatment of diseases or illness caused by *Aspergillus* and *Mucor* spp.

Keywords: Antifungal activity, *Aspergillus*, Extract, *Mangifera indica, Mucor,* Phytochemical.

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INTRODUCTION

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruits and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life [1]. In addition, in some cases, fungi are indirectly responsible for allergic or toxic disorders among consumers because of mytotoxins or allergens. In general, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to harmful effects of pesticides on human health and the environment [2]. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogenic resistant to the products employed, justifies the search for novel active molecules and control strategies [2].

Since antiquity, the plant kingdom provided a variety of compounds of known therapeutic properties, such as analgesic, anti-inflammatories, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world [3-5]. Several have demonstrated in laboratory trials that different plant tissues, such as root, leaves, seeds, and flowers, possess inhibitory properties against bacteria, fungi, and insects [6]. At present, there is a little evidence on the antimicrobial activities of medicinal plants under investigation against phytopathogenic fungi. Medicinal plants are of great important to the health of individuals and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological active on the human body. The most important of these chemical active (bioactive) constituents of plants are alkaloids, tannin, flavonoid, and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes [6].

Mango possesses antidiabetic, antioxidant, antiviral, and anti-inflammatory properties. Other various effects of mango such as antibacterial, antifungal, anti-helmintic, antiparasitic, anticancer, anti-HIV, anti-born resorption, antispasmodic, antipyretic, ant diarrheal, immune modulation, hypolipidemic, antimicrobial, hepatoprotective, and gastroprotective were also reported.

Many fungal species including mold have been known to affect bread which makes this product unhealthy for consumption. *Mangifera indica* is known to possess strong antifungal activity; thus, there is a need to explore the effect of leave extracts from this plant and use it against the bread associated fungi. Plants provide new chemicals entities for the development of drugs against various pharmacological targets as *M. indica* is one of such an alternative that has been used in African tradition. Hence, the need for this study will provide awareness of importance of *M. indica* as an antifungal agent against different fungal species. This research was aimed to isolate different molds associated with bread sold within Bakori metropolis and to determine the phytochemicals constituent as well as antifungal efficacy of *M. indica* leaf extract on some selected fungi isolated from bread.

METHODS

Collection of *M. indica* samples and processing

Leaves of *M. indica* were collected from Bakori metropolis. The taxonomic identity of *M. indica* was confirmed by a botanist (Mr. Musa, D.)

in the Department of Biological Sciences, Federal University Dutsin-Ma, Nigeria.

Preparation of *M. indica* leaf extract

M. indica leaves were washed with distilled water and dried for 2 weeks. The leaves were pulverized into fine powder using a hand mill grinder. About 50 g of the powdered leaves were filled in a thimble filter paper, blocked with cotton wool. A total of 500 ml of ethanol were added into a round bottom flask and then attached to the Soxhlet extractor and condenser on an isomantle. The thimble plant materials were added to the extractor and the solvent was heated using isomantle until it began to evaporates, moving through apparatus to the condenser. The condensate then drips into the reservoir containing the thimble once the level of the solvent reaches the siphon then it poured back into the flask and the cycle rebegin again [7]. The process was run for up to 16 h and filtered on concentrated under the vacuum using rotary evaporator. *M. indica* leave extract was kept in sterile bottle before phytochemical screening and antifungal determination [7].

Preparation of media

The culture media potato dextrose agar (PDA) was prepared in line with the manufacturer's direction.

Collection of fungal samples

Loafs of bread were purchased from four different locations within Bakori metropolis, each of the two was from different manufacturing industries in a sterile polythene bags and kept for 10 days until when there was a visible growth.

Isolation of fungi

The observed fungal masses on the bread surface were scraped using needle and inoculated on PDA plates. The same procedure was carried out for other fungal colonies on various loafs of bread with different colors and the plates were labeled and incubated at 27±1°C for 5 days [8].

Preparation of pure culture

After the incubation, the colonies on the PDA plates were observed, a little was scraped from a well separated colony and placed in the center of another PDA plate and incubated at 27±1°C for another 5 days.

Morphological identification of the fungi

The fungi isolated were identified based on their cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation [9]. The method of Oyeleke and Manga [10] was adopted for the identification of the isolated fungi using cotton blue in lactophenol stain.

The identification was achieved by placing a drop of lactophenol stain on a clean slide by the aid of a mounting needle, where a small portion of the aerial mycelium from the respective fungal culture was removed and placed on the lactophenol stain. The mycelium was spread and the slide was then mounted and viewed under a compound light microscope under ×100 and ×400.

Phytochemical screening

Test for alkaloids

Two drops of Mayer's reagent were added to 0.5 g of *M. indica* ethanol leaf extract in a test tube. Appearance of white creamy precipitate indicates the presence of alkaloids [11].

Test for saponins

A 0.5 g of *M. indica* ethanol leaf extract was mixed with 10 ml of distilled water. The preparation was kept for 15 min with regular shaking. Layer of foam indicates the presence of saponins [11].

Test for cardiac glycoside

A 0.5 g of the leaves extract was weighed and dissolved in 2 ml glacial acetic acid in a test tube. More drop of ferric acid solution was added. A milliliter (1 ml) of concentrated H_2SO_4 was placed in the preparation. A brown at the interface indicates the presence of deoxy sugar characteristics cardenolides [12,13].

Test for flavonoids

A 0.5 g of *M. indica* was weighed and three drops of ferric chloride solution were added. A green or blue color indicates the presence of phenolic nucleus [11].

Test for steroid

A 2 ml of acetic anhydride was added to 0.5 g of *M. indica* extract. A 2 ml of H_2SO_4 was suspended (added), a color changes from violet to blue which indicate the presence of steroid.

Test for tannins

A 0.5 g of *M. indica* extract was mixed with few drops of distilled water. A 3 ml of 10 lead acetate solution was added. A bulky white precipitate indicates the present of tannins [14].

Determination of antifungal activity of *M. indica* extract

Stock solution of ethanol extract of *M. indica* was prepared by dissolving 1 g of the extract in 10 ml of dimethyl sulfoxide. Serial doubling dilution of the stock solution was employed to obtain concentrations of 100 mg/ml, 50 mg/ml, and 25 mg/ml. Antifungal activity of *M. indica* extract was carried out using agar well diffusion method on PDA [15].

PDA was poured into sterile Petri dishes (25 ml) and it was allowed to solidify at room temperature. The fungi (mold) were transferred onto the PDA plates. A sterile cork borer (6 mm) was used to bore three wells in each inoculated plate, then 0.1 ml of different concentrations (100 mg/ml, 50 mg/ml, and 25 mg/ml) of *M. indica* leave extract were separately introduced into the wells. The preparation was incubated at 25°C for 24 h. Zone of inhibition was measured using meter rule and the means were recorded in milliliters [15].

Determination of minimum inhibitory concentration (MIC)

Sterile beakers (10 ml) were used to prepare different dilutions of *M. indica* ethanol leave extracts (100 mg/ml, 50 mg/ml, and 25 mg/ml). MIC of *M. indica* ethanol extract was determined using the broth dilution technique. Three set of sterile test tubes were placed in a test tube rack and labeled. A 4 ml of nutrient broth was dispensed into each tube using a sterile syringe. A 0.1 ml of each standardized inoculum was separately inoculated into sterile 5 ml syringe and 1 ml each of varied concentrations was dispensed in the labeled containing the nutrient broth and fungal suspensions. The test tube was incubated at 25°C for 24 h. The tubes were examined for visible growth of fungi by checking for the turbidity. The dilutions that first show no sign of visible turbidity were taken as MIC [15].

Minimum fungicidal concentration (MFC)

The test tubes with fungal standardized inoculums that show no visible turbidity after incubation were subcultured on PDA plates and incubated at 25°C for 24 h. The plate that shows no visible growth after incubation was considered as MFC [15].

RESULTS

Morphological characteristics of the isolates

Based on morphological characteristics, the cultural appearance, color, and pigmentation of the isolates were studied. Moreover, the shape and color of the fungal spores were also studied. The pigmentation of the fungi includes greenish, glossy white, and blackish, as presented in Table 1.

Phytochemical screening of *M. indica* leaf extract

The result of phytochemical screening of *M. indica* leaf extract was studied. Six different compounds were determined, the compounds that were present include alkaloids, flavonoid, saponins, steroids/terpenes, and tannins, while cardiac glycoside was found to be absent as indicated in Table 2.

Table 1: Morphological characteristics of the fungal isolates

Samples	Pigmentation	Microscopic features	Cultural appearance	Isolate identity
A	Greenish	Non-branched conidiophore	Pink-like green growth	*Aspergillus niger*
D	Blackish	Conidiophores with bulb end carrier	Pink-like black growth	*Aspergillus flavus*
E	Greenish	Non-branched conidiophore	Pink-like green growth	*Aspergillus niger*
F	Blackish	Conidiophores with bulb end carrier	Pink-like black growth	*Aspergillus flavus*
G	Glossy white	Sporangia without rhizoids	Cotton-like white growth spotted with black	*Mucor* spp.
H	Greenish	Non-branched conidiophores	Pink-like green growth	*Aspergillus niger*

Table 2: Phytochemical profile of *Mangifera indica* leaf extracts

Phytochemicals	Availability/presence
Saponins	+
Sterols	+
Flavonoids	+
Tannins	+
Alkaloids	+
Glycosides	–

Key –: Absence, +: Presence

Antifungal activity of *M. indica* leaf extract

M. indica ethanol leaf extract exhibits antifungal effect against the isolated fungi (*Aspergillus* and *Mucor* spp.). The extract was found to inhibit the growth of *Aspergillus niger* at 100 mg/ml, 50 mg/ml, and 25 mg/ml with 13.9 mm, 11.5 mm, and 8.0 mm zone of inhibition, respectively. Similarly, the extract was found to inhibit the growth of *A. niger* at 100 mg/ml, 50 mg/ml, and 25 mg/ml with 13.6 mm, 11.2 mm, and 8.1 mm zone of inhibition, respectively. *Mucor* spp. was found to be resistant at 25 mg/ml while 100 mg/ml and 50 mg/ml showed an activity, as presented in Table 3.

Minimum inhibitory concentration (MIC) of *M. indica* leaf extract

Based on MIC results, *M. indica* was found to be promising against *Mucor spp.* at 50 mg/ml concentration while *A. niger* and *Aspergillus flavus* at 25 mg/ml. However, the extract demonstrates an activity against *Aspergillus spp.* all the tested concentrations. On the other hand, MFC shows no activity against all the identified isolates, as indicated in Table 4.

Table 3: Antifungal activity of *Mangifera indica* leaves extract against *Aspergillus* and *Mucor* spp

Fungi isolates	Zone of inhibition (mm) of different concentration in mg/ml of *Mangifera indica* ethanol leaves extract		
	100 mg/ml	50 mg/ml	25 mg/ml
Aspergillus niger	13.9 mm	11.5 mm	8.0 mm
Aspergillus flavus	13.6 mm	11.2 mm	8.1 mm
Mucor spp.	12.4 mm	8.3 mm	None

Table 4: Minimum inhibitory concentration of *Mangifera indica* leaf extract against the fungal isolates

Fungi isolates	Varied concentrations (mg/ml) of *Mangifera indica* ethanol leaves extract		
	100 mg/ml	50 mg/ml	25 mg/ml
Aspergillus niger	None	None	MIC
Aspergillus niger	None	None	MIC
Mucor spp.	None	MIC	None

None implies; no activity (presence of turbidity), MIC: Minimum inhibitory concentration

DISCUSSION

This study examines the phytochemical profile and antimicrobial activity of *M. indica* ethanol leaves extract against *A. niger, A. flavus,* and *Mucor* spp.

The cultural and morphological characteristics of the isolates confirmed the identity to be *A. niger*, *A. flavus,* and *Mucor* spp. Five phytochemicals, namely, alkaloids, flavonoids, saponins, steroid/terpenes, and tannins were found to be present in *M. indica* ethanol leaves extract. The saponins have detergent properties and exhibit inflammatory properties, alkaloids are plant-derived compound that is toxic or physiologically active and it is of limited distribution in plant kingdom, alkaloids have been reported to be responsible for antifungal activity. Flavonoids are involved in symbiotic nitrogen fixation and floral pigmentation in higher animals, which are similar with the findings of Sahrawat *et al*. [5].

M. indica ethanol leaves extract exhibits antifungal effect against the isolated fungi (*A. niger*, *A. flavus,* and *Mucor* spp.). The extract was found to be favorable in inhibiting the growth of *Aspergillus* spp. at different concentrations (100 mg/ml, 50 mg/ml, and 25 mg/ml).

The results of MIC and MFC of *M. indica* ethanol extract indicate that *M. indica* was found to be promising against *Mucor* spp. at 50 mg/ml concentration, while *A. niger* and *A. flavus* were at 25 mg/ml. Different potential pharmacological actions, including antimicrobial (especially against bacteria and filamentous fungi) effects, were corroborated in the previous studies [16-18]. However, a few numbers of yeasts (*C. albicans, C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus albidus,* and *Saccharomyces cerevisiae*) were tested against mango by-products. The antifungal properties have been demonstrated on *C. albicans* using different parts of the plant; stem bark [19], leaves, rind [20], seeds [21,22], and unripe dried pulp (amchur) [23]. Furthermore, Stoilova *et al*. [24] and Singh *et al*. [16] showed the antifungal activity of mangiferin and its analog compounds. Moreover, in some works, the MICs were determined and the results have been diverse. Apparently, the disparity data could be attributed to the different *C. albicans* strains tested (some of them were not reference strains), the samples origin (leaves, fruit, etc.), and the extraction process (especially the type of solvent) of the extracts; MICs = 0.08 mg/ml stem bark [19]; 7.5–12.5 mg/ml leaves; 7.5–17.5 mg/ml rind [20]; and 0.3 mg/ml amchur [23].

CONCLUSION

M. indica leaf extract demonstrates a promising antifungal activity against the three isolates of the fungi, this is due to the massive collection of phytochemicals such as alkaloid, flavonoid, saponins, steroid/terpenes, and tannins. Therefore, *M. indica* leaves extract can be used in the treatment of diseases or illness caused by *A. niger, A. flavus,* and *Mucor* spp. There is a need to focus on molecular method of identifying the fungi instead of relying on morphological characteristics which is time consuming and difficult to obtain the accurate results.

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