Antifungal activity of "Mahogany" (*Khaya senegalensis*) leaf extract against some selected fungi

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Abstract

Effect of leaf aqueous extracts of *Khaya senegalensis* were determined on disease causing fungi. This work aimed to determine the antifungal effect of *Khaya senegalensis* leaf extract on the growth of some selected fungi. In this study, the phytopathogenic fungi isolated from the spoiled bread are identified based on morphological and cultural characters. The identification of the fungi was confirmed by pathogenicity tests, the organisms were found to be *Mucor* spp. and *Rhizopus* spp. Leaf aqueous extracts of different concentrations (100, 200, 300, 400 and 500 mg mL^{-1}) of *Khaya senegalensis* were added to the growth media prior to inoculation. The aqueous extracts have inhibited the mycelial growth of the fungi, and this effect gradually increased with an increase in the concentration. It could be emphatically concluded that the tested plant extract can effectively control fungi affecting. This makes it potential biofungicide for the management of fungi associated with bread as it is cheap and environmentally safe due to their fungicidal and fungitoxic ability.

Keywords: Antifungal, biofungicide, efficacy, fungi, leaf extract.

Resumo

O efeito de extratos aquosos de folhas de *Khaya senegalensis* foi determinado sobre fungos causadores de doenças. Este trabalho teve como objetivo determinar o efeito antifúngico do extrato da folha de *Khaya senegalensis* no crescimento de alguns fungos selecionados. Neste estudo, os fungos fitopatogênicos isolados do pão estragado são identificados com base em caracteres morfológicos e culturais. A identificação dos fungos foi confirmada por testes de patogenicidade, os organismos encontrados foram *Mucor* spp. e *Rhizopus* spp. Extratos aquosos de folhas de diferentes concentrações (100, 200, 300, 400 e 500 mg mL-1) de *Khaya senegalensis* foram adicionados ao meio de crescimento antes da inoculação. Os extratos aquosos inibiram o crescimento micelial dos fungos, e esse efeito aumentou gradativamente com o aumento da concentração. Pode-se concluir enfaticamente que o extrato vegetal testado pode efetivamente controlar os fungos que afetam. Isso o torna potencial biofungicida para o manejo de fungos associados ao pão, pois é barato e ambientalmente seguro devido à sua capacidade fungicida e fungitóxica.

Palavras-chave: Antifúngico, biofungicida, eficácia, fungos, extrato de folhas.

Resumen

Se determinó el efecto de extractos acuosos de hojas de *Khaya senegalensis* sobre hongos causantes de enfermedades. Este trabajo tuvo como objetivo determinar el efecto antifúngico del extracto de hoja *de Khaya senegalensis* sobre el crecimiento de algunos hongos seleccionados. En este estudio se identifican los hongos fitopatógenos aislados del pan podrido con base en caracteres morfológicos y culturales. La identificación de los

hongos se confirmó mediante pruebas de patogenicidad, los organismos resultaron ser *Mucor* spp. y *Rhizopus* spp. Extractos acuosos de hojas de diferentes concentraciones (100, 200, 300, 400 y 500 mg mL^{-1}) de *Khaya senegalensis* se agregaron a los medios de crecimiento antes de la inoculación. Los extractos acuosos han inhibido el crecimiento micelial de los hongos, y este efecto aumenta gradualmente con el aumento de la concentración. Se podría concluir enfáticamente que el extracto de planta probado puede controlar eficazmente los hongos que afectan. Esto lo convierte en un potencial biofungicida para el manejo de hongos asociados al pan ya que es económico y ambientalmente seguro debido a su capacidad fungicida y fungitóxica.

Palabras clave: Antifúngico, biofungicida, eficacia, hongos, extracto de hoja.

1. Introduction

Plants are one of the most important sources of medicines, agrarian science, biological science and biotechnology (Menezes Filho et al., 2022). Medicinal plants are rich in special metabolites which are potential sources of drugs and of therapeutic importance. Finding healing power in plants is an old idea. People would-over long applied poultices and imbibed infusions of thousands of indigenous plants dating to prehistory. Human disease management in Nigerian history also provides evidence of the relationship of plants and medicine (Ayandele and Adebiyi, 2007; Ru-yong et al., 2007). The medicinal flora in the tropical eco-region has a preponderance of plans, they provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as special metabolites (Bashir et al., 2014; Bashir et al., 2021).

Khaya senegalensis A. Juss. (Meliaceae family) commonly known as African mahogany, is a popular medicinal plant among the Nigeria folks. It belongs to the family Meliaceae (Mahogany). *K. senegalensis* is a tree with shiny foliage up to 25 m or more with exfoliating barks, young branches with dark, grayish-brown lenticels and leaves of 15-60 cm or more. It has pinnate leaves, glabrous with 6 to 12 alternate or opposite elliptical oblong leaflets. At the flowering, *K. Senegalensis* twigs is carried at their end panicles of small white flowers consisting of successive whorls of four flora part. Its fruits are capsules with thick and woody seed coat. *K. senegalensis* seeds have been reported to contain about 67% oil content by weight (Ademola et al., 2004).

The *Rhizopus* spp. have also emerged as important causes of morbidity. Different species of fungi are responsible for destroying major agricultural commodities that affect before and after harvest, and also animal product such as meat, milk, eggs, and breed, can become contaminated by fungal growth, mycotoxins (Wilkoff et al., 2002), and mortality in immune-compromised patients (Paterson et al., 2000) and is an important cause of opportunistic respiratory and disseminated infection in other immunocompromised patients (Benjamin et al., 2002). Aflatoxins are still recognized as the most important mycotoxins. The expression of aflatoxin-related disease is influenced by factors such as age, nutrition, sex species and the possibility of concurrent exposure to other toxins. The main target organ in mammal is the liver so aflatoxicosis in humans includes limited availability of food, environmental conditions that favor mold growth on foodstuffs, and lack of regulatory system for aflatoxin monitoring and control (Henry et al., 2011; Williams et al., 2010).

Several published reports have shown the effectiveness of traditional herbs against microorganisms (Menezes Filho et al., 2020a & b; Menezes Filho et al., 2021; Obafemi, 2006). Medicinal plants contain physiologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments (Adebanjo et al., 2012). As a result, plants are one of the bedrocks of modern medicine. The screening of plant extract and natural product for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents as well as serving drugs discovery from natural products for primary leads compounds. *K. senegalensis* plants and some other plants are used in many African countries by traditional medicinal practitioners for the treatment of various ailments including fungal and bacterial disease (Umeh et al.,2005).

This study was aimed at screening the antimicrobial activities of *Khaya senegalensis* leaf extracts for the possibility of enhancing the use in medicine against pathogenic fungi, it is a plants commonly used by the local people in Nigeria for the treatment of dysentery, mucous diarrhea and wound infection (Magbool et al., 2011).

2. Materials and Methods

Reagents and equipments

Sabouraud Dextrose Agar (SDA), ethanol (Biomax Scientific Bhd. LTD. Malaysia), gentamycin, lactophenol, distilled water, needle, *Petri* dishes, slide, cover slip, compound light microscope, incubator, scissors, scalpel, and parafilm (Biomax Bhd. LTD. Malaysia).

Study area

The study was carried out at the new Biology laboratory, Department of Biological Sciences, Federal University Dutsin-Ma, Nigeria. Dutsin-Ma is located in northern guinea savannah zone of Nigeria coordinates (latitude 12^{o} 27' 18' N and longitude 7^{o} 29' E).

Sample's collection

The leaf of *K. senegalensis* (Herbarium voucher No. 900181) were collected in the morning (7:30am) from Kadangaru area, Dutsin-Ma town in May, 2019. The samples were cut into small pieces with a clean scalpel and air-dried under room temperature. hey were later ground into fine powder using laboratory mortar and pestle in the Department of Biological Sciences, Faculty of Life Science, Federal University Dutsin-Ma. The powdered sample were stored in clean, dry, air-tight bottles and kept in a cool, dry place until require for used.

Extraction procedure

50 g of the powdered leave were weighed and poured into 500 mL conical flask in which 200 mL of distilled water was added (Bashir et al., 2018b). The mixture was kept for 12 hours with constant agitation at 30 minutes' intervals. The extract was filtered using Whatman No.1 filter paper. The filtrate was concentrated in a vacuum and transferred to water bath in order to remove the moisture. The semi-solid extract obtained was stored in a refrigerator (4 °C) for further use Bashir et al., 2018b.

Preparation of Sabouraud Dextrose Agar (SDA)

The dry ingredient for SDA was measure according to manufacturer instruction using a weighing balance. It was poured into a conical flask and the proper amount of distilled water was added and conical flask was covered with a foil paper. It was shaken well and placed on a hot plate to help dissolved the content, stirring gently in the process to prevent it from burning. It was then sterilized in an autoclave at 120 °C for 15 min. After cooling down a bit (for about 30 min.) Antibiotic (Gentamycin 0.025 g) was added to prevent the growth of bacteria, the media was then dispensed aseptically into *Petri* dish and allowed to solidify (Williams et al., 2010).

Preparation of pure culture

After 7-10 days, pure culture was prepared from the primary culture which appeared to be mixed when transferring the fungi, bent inoculating needle was used to cut a small block of agar containing mycelium from the primary culture. The agar piece was placed on the new sterile medium. The needle was flamed before and after use. The culture was covered, labeled and incubated at room temperature (25 ± 1°C) for another 7-10 days (Kutawa et al., 2021; Tijjani et al., 2018a, b, & c).

Isolation and identification of pathogens

A drop of lactophenol cotton blue was placed on a clean slide. A small piece of mycelium from the isolate was removed using two inoculating needles on either hand and placed on the stain, teasing out the mycelium carefully with the needle. It was then covered with a cover slip carefully to avoid air bubbles and was observed under high power (40x) of the microscope (Bashir et al., 2017; Bashir et al., 2018a&b). This was done for all isolates. The identification was based on features such as organization of hyphae (pencil shaped, spiral, pyriform, septations etc.), microconidia and macroconidia (tear shaped, drop like, spherical, in bunches, abundance or rare etc.). The fungi were identified as *Mucor* and *Rhizopus* species by Mr. Kabir D., and were deposited in the mycological bank of the Department of Biological Sciences, Federal University Dutsin-Ma, Nigeria in July,2019. The percentage inhibition of mycelial growth by each extract was computed using formula (Bashir et al., 2020).

$$I = 100\times(C - T)/C$$

Where: I = percentage inhibition of mycelial growth; C = mycelial growth of fungus in control plate; T =

mycelial growth of fungus in the treatment.

Statistical analysis

The experimental design was based on completely Randomized Design (CRD), and the data obtained were analyzed based on one-way analysis of variance (ANOVA).

3. Results

The result showed that the aqueous leaf extract of *K. senegalensis* have inhibitory effect on *Rhizopus spp.* and *Mucor spp*. Table 1 shows the number of colonies found in the mixed culture. The fungal load for isolate 001 was 3.60 x 10^2, 2.44 x 10^2, 1.30 x 10^2, 7.1 x 10^1 and 4.0 x 10^1 at concentration of 10^{-1}, 10^{-2}, 10^{-3} 10^{-4} and 10^{-5}, respectively. While isolate 003 was having fungal load of 2.60 x 10^2 1.02 x 10^2 8.20 x 10^2 5.0 x 10^1 and 2.7 x 10^1 at concentration of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively.

Table 1. Fungal load on bread sample obtained from mixed cultures.

Samples	**Fungal load**				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Isolate 001	3.60 x 10^2	2.44 x 10^2	1.30 x 10^2	7.1 x 10^1	4.0 x 10^1
Isolate 002	3.70 x 10^2	2.90 x 10^2	1.60 x 10^2	1.02 x 10^2	7.0 x 10^1
Isolate 003	2.60 x 10^2	1.02 x 10^2	8.2 x 10^1	5.0 x 10^1	2.70 x 10^1

For *Rhizopus*, the highest inhibitory percentage was 75% at 500 mg mL^{-1} of the extract. It was followed by 64.1%, 63.2%, 54.7% and 50.6% at 400 mg/ml, 300 mg/ml, 200 mg/ml and 100 mg/ml, respectively. The Mycelial growth was found to be 2.91 mm, 2.67 mm, 2.17 mm, 2.12 mm and 1.42 mm at concentration of 100, 200, 300, 400 and 500 mg/mL respectively, while the control was having 5.89 mm as presented in (Table 2). It was alsoobserved that inhibitory effect increased with the increased in concentration of leaf extract.

Table 2. Inhibition of mycelial growth of *Rhizopus spp.* with aqueous plant extracts.

Extract concentration (mg mL^{-1})	**Mycelial growth (mm)**	**Inhibitory percentage**
100	2.91±0.21	50.6%
200	2.67±0.87	54.7%
300	2.17±0.93	63.2%
400	2.12±1.10	64.1%
500	1.42±1.00	75.9%
Control	5.89±0.33	-

-= absent, ± = standard error

For *Mucor*, the highest inhibitory percentage was 64% at 500 mg mL^{-1} of the extract. It was followed by 51.5%, 37.5%, 23.4% and 9.4% at 400 mg/ml, 300 mg/ml, 200 mg/ml and100 mg/ml respectively. The Mycelial growth was found to be 3.87 mm, 3.27 mm, 2.67 mm, 2.07 mm and 1.5 mm at concentration of 100, 200, 300, 400 and 500 mg/ml respectively, while the control was having 4.27 mm as presented in (Table 3). It was also observed that inhibitory effect increased with the increased in concentration of leaf extract.

Table 3. Inhibition of mycelia growth of *Mucor spp* with aqueous extract.

Extract concentration (mg mL^{-1})	**Mycelial growth (mm)**	**Inhibitory percentage**
100	3.87±0.23	9.4%
200	3.27±1.12	23.4%
300	2.67±1.11	37.5%
400	2.07±0.23	51.5%
500	1.5±0.77	64.9%
Control	4.27±0.45	-

-= absent, ± = standard error

4. Discussion

The mycelial growth inhibition of fungi by the leaf aqueous extracts of K. *senegalensis* investigated in this study indicated that, antifungal activity showed by the tested plant extract had inhibitory effects on the mycelial growth of *Rhizopus spp* and *Mucor spp.* These results further revealed that antifungal activities of the extracts were enhanced by increasing the concentration from 100 mg mL^{-1} to 500mg/mL; hence the inhibition activitiesof the extracts were concentration dependent.

This is in agreement with the report of Ilondu (2012) and Bashir et al. (2021) who indicated that increase in the antifungal activities had corresponding increase in concentration of plant extract. *K. senegalensis* exhibited fungitoxic effect by inhibiting the mycelial growth against the *Rhizopus spp.* and *Mucor spp*. The antifungal activity of *K. senegalensis* conforms to the results of Omar et al. (2003) and Bashir et al. (2021) that the extract is very effective in inhibiting the growth of *Fusarium moniliforme, Aspergillus flavus* and *Aspergillus niger.*

The fungitoxic properties of *K. senegalensis* could be attributed to the presence of saponin and alkaloid, chemical components which has been identified as antifungal agents in the plant (Kubmarawa et al., 2008; Marinho et al., 2022). The fungicidal effects of plant extracts on different pathogens of plants have been widely reported. However, the differences in the efficacy of the extract could be attributed to the differences their active ingredients (Okigbo et al., 2006). Major compounds of plant extracts are phenols, flavonoids, alkaloids, quinones, saponins, tannins and sterols (Halama & Van Haluwin, 2004) and their fungicidal or fungistatic properties against the various plant pathogens have been established. These products might either have direct inhibitory effects on pathogens, exhibiting fungicidal or fungistatic properties.

It was very obvious that increase in concentration of plant extract have caused decrease in fungi mycelial growth. This may be due to presence of large quantities of antifungal substances at higher concentration in the plant materials. This is in accordance with the work of Bashir et al. (2021) where the occurrence of fungi from the bread was significantly reduced after the addition of 5% mango leaves extract, when compared to the previous concentrations used i.e. 1% and 3%. Also when leaves extract of *Lantana camara*, *Cannabis sativa*, and *Cymbopogon pendulus* were tested against *Colletotrichum gloeosporioides*, the causal agent of brown blight tea, they observed that, as the concentration increases, there was an increased inhibition in spore germination of the fungi.

5. Conclusions

The aqueous extract of leaf of *K. senegalensis* has antifungal activity and could provide a therapeutic agent for the treatment of ailments caused by *Rhizopus spp*. The extract from leaf of *K. senegalensis* was found to be more effective on *Rhizopus spp*. mycelia growth inhibition than in *Mucor spp*. Although, the efficacy of the extract was found to vary with increase in concentration. More research should be conducted that will further elucidate and characterized theactive component and their mechanism of action. It is also recommended that leaf extract of *K. senegalensis* should be produced in commercial quantity for the treatment of ailments associated with *Rhizopus* and *Mucor spp*.

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