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Antifungal Potential of Black Mangrove Leave Extracts on Isolates of Smoked Bonga Fish Sampled from Oju-Ore and Iyana Markets in Ota, Ogun State, Nigeria

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ABSTRACT: The contamination of dried fishes by fungi poses serious threat to food safety and public health. This study was carried out on fungal contaminants found in smoked Bonga fish and to determine if there are any antifungal properties in the ethanol extract of Black Mangrove leaves. To achieve this, the study made use of six bonga fish samples purchased from two different markets, with six samples from three different retailers in each market, making a total of thirty-six samples. The antifungal properties of Black Mangrove *(Avicennia germinans*) were determined; the leaves were dried in the open air, followed by blending and extraction using absolute ethanol. Mycological examination of samples revealed the prevalence of seven types of fungi namely, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus* sp, *Alternaria, Candida* sp, *Mucor* sp. Concentration of 1% and 0.1% was applied, using the agar well diffusion method. The 1% ethanol extract showed very small zone of inhibition on the fungal isolates. *Rhizopus, A. niger, A flavus, A. Fumigatus, Alternaria* sp, *Candida* sp, *Mucor* sp showed zones of inhibition 2mm, 1mm, 0mm, 1mm, 0mm, 1mm, 2mm, respectively. The 0.1% ethanol extract showed no zone of inhibition. This showed that the extract had little or no antifungal effects on the isolates.

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Fish is extremely nutritious, this is due to the rich protein content. It has been reported that fish provides to over 400 million persons in the underprivileged countries of Africa and Asia more than half of their protein consumption (Obiero *et al.*, 2019). Also, Cojocaru *et al*. (2022) stated that freshwater fish is one of the significant sources of micronutrients, protein, and offer food security and to numerous tropical forest-dwelling local populations. Conversely, fish is a fitting medium for microbial growth, if poorly handled (Oparaku and Mgbenka, 2012). In Nigeria, fish is more consumed than meat, this is because it is cheaper, relatively available, makes up about 40% of the animal protein intake and contains a substantial amount of vitamin A, B and E, iodine and oils containing polyunsaturated fatty acid (Eyo, 2001). Fish products deteriorate owing to the growth of microorganisms and other non-microbial activities such as lipid oxidation (Martin, 2008). An increase in the surrounding temperature creates favourable conditions for microorganisms to thrive, which lowers the quality of fish and its possible shelf life, resulting in food loss (Abolagba and Uwagbai , 2011). Food preservation typically involves steps that inhibit the growth of microorganisms, either by adding ingredients that prevent growth or by modifying storage conditions through freezing or drying (Akise *et al.*, 2013). Because the product is preserved, smoke dried fish may have a long shelf life because the storage method used inhibits the microflora from growing. However, Sani *et al.*, (2016) reported several cases of human gastroenteritis, severe diarrhoea and

food poisoning outbreaks after smoked fish consumption. Also, fungi or their byproducts could be the cause of some food-borne diseases. Alternaria, Aspergillus, Candida, Fusarium, Mucor, and other pathogens are examples of fungi that contaminate food (Tomsikova, 2002). Unhygienic food workers who work in eateries have a higher risk of spreading enteric pathogens. Meanwhile, in rural areas, many of the plant materials used in traditional medicine are easily accessible and comparatively more affordable than modern medicine. A significant reservoir of aromatic food and medicinal plants can be found in African flora in general. By definition, a plant is considered to be medicinal if one or more of its components, such as a compound that can be used as a precursor to synthetic antimicrobials or for therapeutic purposes (Sofowora, 1984). Generally, plants generate a large number of secondary metabolites, which are a major source of microbicides, pesticides, and numerous pharmaceuticals. It has been reported that therapeutic plants are essential to the pharmacopoeia (Badiaga, 2011). Antimicrobial compounds are common in medicinal plants (Mahesh and Satish, 2008). Furthermore, the use of traditional methods for fish processing and preservation is predominant in Africa (Daramola *et al*., 2020). This includes the preservation of fish and fish products with local materials such as extracts from plants and related sources. The antifungal activities of some plants have been reported by different researchers (Farombi, 2003; Oyelami *et al*. 2003; Mahesh and Satish 2008). Shanmugapriya *et al*. (2012) showed that the saponin content is present in leaf of *Avicennia marina* and absent in leaf of *Avicennia officinalis.* Mangrove foliar extracts have been proven through scientific examination to have great potential against microbial pathogens (Rastegar and Gozari, 2017) and specifically Padmakumar and Ayyakkannu (1997) stated that mangrove plants possess antibacterial, antifungal and antiviral qualities. However, the advent and acceptance of antibiotics had caused the use of plant derivatives as antimicrobials to drastically decline. Therefore, the aim of this study was to evaluate the antifungal potential of black mangrove leave extracts on isolates of smoked bonga fish from Oju-Ore and Iyana markets in Ota, Ogun State, Nigeria.

MATERIALS AND METHODS

Collection of fish samples: Samples of dried *Ethmalosa fimbriata* (Bonga fish) werenobtained from Oju-Ore and Iyana markets in Ota, Ado-Ota Local Government Area, Ogun State, Nigeria. From the two markets, six samples from three different retailers in each market were purchased, making a total of thirty-six samples. The samples were collected aseptically with Ziploc bag. The samples were transported to the laboratory at room temperature and analysis was carried out in less than 24 hours after fish collection.

Sample preparation: The method described by Sulieman and Mustafa (2012) was modified for the purpose of counting and identifying fungi from the smoke-dried fish samples, including yeasts and moulds. To create a homogenate sample, 10 grams of dried fish were combined with clean distilled water. The suspension was serially diluted from 10^{-1} to 10^{-3} per milliliter in sterile saline. A quantity of 0.1ml was inoculated onto sterile Potato Dextrose Agar from the 10^{-3} dilutions (PDA). For 48 to 72 hours, the plates were incubated at 25–28°C, and fungal colony formation was observed. The number of yeasts and molds was counted using a colony counter and expressed in cfu/g. For the purification of fungal isolates, the hyphal tip technique procedure (Bazyani and Saadullah, 2018) was used. The macroscopic identification of mould isolates was done by observing the morphology, colouration, and growth pattern of the colonies (Denning, 2005 in: Pitt and Hocking, 2009). For microscopic fungi detection, lactophenol cotton blue staining was applied to a wet mount of the mould.

Samples Collection for Black Mangrove Leaves and Processing: The Black Mangrove (*Avicennia germinans*) leaves was purchased from Mangrove vegetation of University of Lagos Lagoon front, pulverized into powder before transporting to the Bells University Biology laboratory. The collected Avicennia germinans leaves were thoroughly washed in clean water in order to remove of any dirt, sand, or insect droppings prior to actually drying. The leaves were allowed to air dry at room temperature before being pulverized into powder. After five days of extraction with 2.5 litres of ethanol in hot and cold water and 222 grams of blended leaves, the supernatant was filtered through No. I Whatman filter paper and the residue was discarded. The crude ethanol, hot and cold water were poured into beakers then concentrated at 60^0C in a water bath.

Agar Preparation: To prepare 20 plates, 16 grams of Potato Dextrose Agar was suspended in 400ml of distilled water. The mixture was then sterilised by autoclaving at 15 lbs pressure at 121℃ for 15 minutes. Thereafter, Chloramphenicol was added to inhibit bacterial growth on the plate at 2% of the total agar solution.

Isolation of the Microorganisms: Using a sterile laboratory mortar and pestle, the bonga fish that were collected for the study were ground into a semi-powdery form. There were six conical flasks placed

out, each holding 90ml of distilled water. Ten grams of the material were weighed, dissolved in 90 ml of distilled water in a test tube 1, and thoroughly shaken. One millilitre of the solution was taken out of the test tube and and transferred into test tube 2, it was shaken properly and same processes were repeated for test tubes 3, 4 and 5. Samples of 0.1 millilitre was taken from each test tube and placed in a Petri plate containing primed Potato Dextrose agar (PDA). The inoculated dishes were covered and incubated for 2 to 7 days at room temperature (28°C–30°C). The serial dilution was done to disperse the fungal inoculum load in the solution to ease isolation.

Identification of Fungal Isolates (using slide culture technique: Procedure: The sterile U-shaped glass rod was inserted into the petri dish after being sterilised and aseptically with a set of sterile forceps. A plate of prepared Potato dextrose agar was gently flamed before a 5mm square piece of the medium was cut from it. The scalpel was inserted, and an agar cube was gently and aseptically transferred to the centre of the slide. The colony of the fungi to be analysed was inoculated onto each of the four sides of the agar using a sterile wire loop. On the top of the inoculated agar was put a sterile cover-slip. The petri plate was moistened by adding one (1ml) of sterile distilled water to it. The petri dish was covered and incubated at room temperate for 4 - 5 days.

RESULTS AND DISCUSSION

A total of seven species of fungi were isolated from the from the fish samples. After isolation, the species of fungi were identified. The morphological characteristics were identified, both microscopically and physically; as shown in table 1. Meanwhile, total colony forming unit of each sample is presented in table 2 and the phytochemical screening of extract of *Avicennia germinans* is shown in table 3. The zone of inhibition for 1% concentration of ethanol extract and the zone of inhibition for 0.1% concentration of ethanol extract are presented in table 4 and table 5 respectively. Furthermore, while the percentage prevalence of isolated fungi from Oju-Ore market is shown in figure 1, percentage prevalence of isolated fungi from Iyana market in figure 2. Akande and Tobor (1992) and cited by Sani *et al.* (2016) reported that the environment where fishes were displayed in markets are generally unhygienic and could constitute another pathway for microbial contamination. Hence, the contamination of smoked Bonga fish by various species of fungi, as observed in this study and many other earlier studies is not surprising. It was observed that retailers often place the samples of smoke-dried fish next to heaps of dirt in open trays, which supports contamination and fungal proliferation through air droplets.

Table 1: Identification of the Isolated Fungi

Isolates	Identification	Characteristics
Isolate A	*Rhizopus* sp	They are identified by the presence of stolons and coloured rhizoid, as well as the formation of pophysate, columellate, multi-spore, generally globose sporangia from nodes right above the rhizoid.
Isolate B	*Aspergillus niger*	Colonies are made up of a thick layer of dark brown to black conidial heads that cover a compact whitish yellow basal felt. Conidiophores have smooth walls that darken toward the vesicle.
Isolate C	*Aspergillus flavus*	Yellow-green colony, Radiate heads on basal mycelium and columns in aerial mycelium. Microscopic features include long conidiophores, rough in distal part, biseriate head with globose smooth conidia
Isolate D	*A. fumigatus*	Presence of green spiked conidia. Presence of dark brown colonies. Radiate heads on basal mycelium and columns in aerial mycelium. Microscopic features include long conidiophores
Isolate E	*Alternaria*	Presence of grey green septate hyphae. Reverse side of the colonies appear black. Septate conidia also appear dark coloured
Isolate F	*Candida spp*	They appear creamy to white colonies on the agar plate. Slightly raised from the surface. Microscopically, they stain gram positive. Cells appear in oval shape.
Isolate G	*Mucor*	Pale greyish brown colonies, sporangiophores are branched

The fungi isolates are important opportunistic pathogens of human and animal health importance (Akwuobu *et al.* 2019). Fungal infections have contributed to the increase of life-threatening systemic fungal infections in recent years (Perea and Patterson 2002). When contaminants in smoke-dried fish, such as some species of Aspergillus and Candida are consumed, the danger of mycotoxins being produced increases and can cause gastrointestinal and metabolic disturbances (Martin, 2008). Meanwhile, only strains of *Aspergillus flavus* presented aflatoxigenic producing potentials, among the moulds isolated by Job *et al.* (2016). Candida, Mucor, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*, Alternaria, and Rhizopus were among the seven fungal isolates identified during this study. In the market samples, Candida species dominated the mycoflora. The majority of the fungal isolates present

in the smoked fish are characteristic of soil and water habitats.

Table 2: The total colony forming unit of each sample

Sample Point/Code	Total Colony Count (cfu/g)
IY1	3.2×10^4
IY2	2.7×10^4
IY3	5.0×10^2
OJ1	3.7×10^4
OJ2	1.2×10^4
OJ3	4.4×10^2

IY= Iyana Market OJ= Oju-Ore Market

Table 3: Phytochemical Screening of Extract of *Avicennia germinans*

Chemical constituents	Status
Saponin	+

KEY: + = present; - = absent

Table 4: Zone of inhibition for 1% concentration of Ethanol Extract

Isolates	Zone of inhibition(mm)
Rhizopus sp	2
Aspergillus niger	1
Aspergillus flavus	0
A. fumigatus	1
Alternaria sp	0
Candida sp	1
Mucor sp	2

Table 5: Zone of inhibition for 0.1% concentration of Ethanol Extract

Isolates	Zone of inhibition(mm)
Rhizopus sp	1
Aspergillus niger	0
A. flavus	0
A. fumigatus	0
Alternaria sp	0
Candida sp	0
Mucor sp	0

There have been reports of Candida growth in mesophilic habitats like rotting vegetation, soil, and plants (Schuster *et al*., 2002). In general, fungi thrive in humid environments, where they can grow and survive under optimal conditions. Fungi are significant destroyers of fish during storage making them dangerous for human consumption Odogwu *et al.* (2021). Previous mycological studies have been reported in on smoke-dried fish sold in markets in different parts of Nigeria (Job *et al*., 2016).

Also, in the works by Osibona *et al*, (2018) and the one carried out on Bonga fish in Epe community (Adetimehin *et al*., 2019), the isolated fungi were quite similar, having as much as four similar isolates. These isolates include *Aspergillus flavus*, *Alternaria mould, Mucor and Rhizopu*s sp.

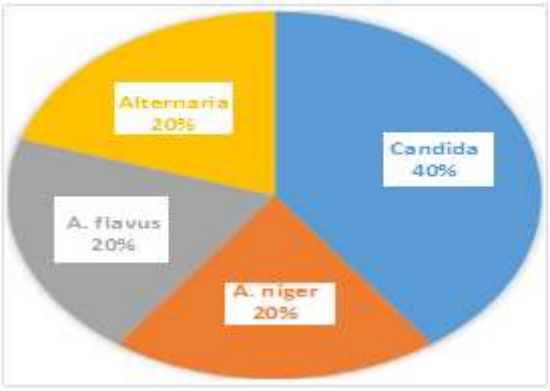

Fig 1: Percentage prevalence of isolated fungi from Oju-Ore market

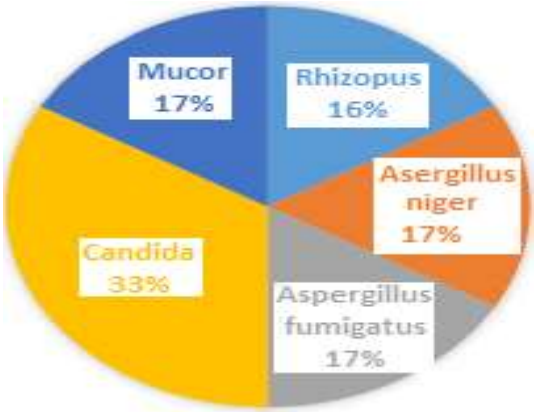

Fig 2: Percentage prevalence of isolated fungi from Iyana market

The fungi isolated from the two different markets also show huge similarities, this is due to the same relative humidity, handling, storage and environmental conditions (both markets are close to the road). The outcome of this study showed that mangrove plant extracts did not possess antifungal potential against the tested fungal strains as the 1% ethanol extract showed very small zone of inhibition on the fungal isolates while the 0.1% ethanol extract showed no zone of inhibition. Inactivation of enzymes, alteration of target sites, reduction in intracellular drug accumulation or ineffective application of extract concentrations are just a few examples of the potential resistance mechanisms the fungal strains may have (Schwarz and Noble, 1999). *Conclusion:* The current research found that different mycoflora, some of which are common toxigenic fungi of importance to human and animal health, are present in the bonga fish sold in open markets in Oju-Ore and Iyana. In view of the aforementioned, the fishmongers and marketers are encouraged to adopt better preservation methods and prevent poor handling, processing and storage; to forestall fungi contamination. Also, fish handlers and customers need to be educated of the dangers that fungi and mycotoxins contaminated fish products pose to their health.

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