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Antimycotic activity of some medicinal plants on aquatic fungi

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ABSTRACT

This study shows the medicinal plant sources commonly used against various aetiological agents and recently being incorporated into some pharmaceutical products. Various plant sources used for the purpose of this study include Azadirachta indica, Ocimum gratissimum, Jatropha species and Carica papaya. Fungal species isolated from aquatic sources during the study include Candida species, Rhizopus spp, Rhizomucor spp, Mucor spp, Aspergillus spp and Penicillium spp. Fungal species such as Candida spp, Rhizopus spp and Mucor spp were found to be common to the three forms of water sources used, that is, the fast moving water, the slow moving water and well which is relatively stagnant. The presence of fungal species in the sampled water sources may be indicative of plant debris contamination from other environmental sources such as soil. The evaluation of the antimycotic properties of these plant extract determined through the antifungal activity from these sources showed their potency against most of the fungal isolates with different levels of growth inhibition. Out of the four plant sources tested, extract from Azadirachta indica was found to be most active with inhibition zone greater than 15mm and total growth inhibition in some instances against the fungal isolates while Carica papaya extracts has lowest activity. This signifies that the active medicinal plant source can be optimized for future antimycotic therapy in diseases control and may solve some antimicrobial resistance problems being products from natural origin.

Key words: Antimycotic, Aquatic, Fungi, Medicinal plants

INTRODUCTION

Plants are natural resources that provide a source of inspiration for novel antifungal compounds based on plant components that contribute largely to human health and safeguard against infections. A host of contaminants from human activities and infiltration of pollutants from our ecological environment contributes to complexity of various forms of microorganisms including saprophytic and pathogenic fungal species in our water resources. Hence, it is of importance to assess the attributes of this group of organisms. Urbanisation, economic growth, and climate change have increased pressure on freshwater resources making biodiversity to give way to the increasing demands of a growing human population. The adverse impacts on aquatic ecosystems include habitat fragmentation, eutrophication, habitat loss, and invasion of pathogenic as well as toxic species [19, 21].

Microorganisms, in particular fungi, possess enzymes capable of degrading highly polymeric substances [9]. During the cold season (autumn, winter and spring), filamentous fungi account for over 90 to 99% of total microbial biomass in emergent macrophytes and riparian leaf litter and their secondary production is one to two orders of magnitude higher than the bacterial production [10, 18]. Additionally, productivity will also be limited by ecological interactions such as competition and mycotrophy [18].

Plant possesses various active substances for biocontrol. They play dual role in the development of new drugs being the base for the development of medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases [11]. Traditional sanitation and disinfection processes using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [30]. They provide an alternative means of controlling fungi since continual use of fungicide has been found to result in the development of resistant strains by previously susceptible species. Various plant sources used for the purpose of this study include *Azadirachta indica*, *Ocimum gratissimum, Jatropha* species and *Carica papaya*.

Azadirachta indica [Neem] is widely used for medicinal purposes in various communities in Nigeria and other parts of the world. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem –a tree for solving global problems'. The advancement of neem research has earlier been documented [13]. Since the early report by Siddiqui in 1942 on the isolation of nimbin, the first bitter compound isolated from neem oil, more than 135 compounds have been isolated from different parts of neem. The compounds from neem have been divided into two major classes: isoprenoids and others18. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and Csecomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins [amino acids] and carbohydrates [polysaccharides], sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc [13]. Some of these compounds such as Gedunin have been established to possess antifungal activities against some fungal species [12].

Ocimum gratissimum belongs to the Lamiaceae family, which has close to 252 genera and 6700 species [16] most of which are used as medicine [17, 26]. The leaves are often hairy and posses epidermal glands which secrete volatile oils giving characteristic scents to many of the species. Under *in vitro* conditions, the oils from leaves, seeds, flowers and roots of *Ocimum* species have shown to have antibacterial activity against many Gram positive bacteria. Furthermore, *O. gratissimum* L. contains an array of secondary metabolites dominated by two classes of compounds: flavornoids and terpenoids including monoterpenoids, sesquiterpenoids, diterpenoids and iridoid glycosides [29].

Jatropha species belong to the family *Euphorbiaceae* and is commonly called physic nut, purging nut or pig nut. *Jatropha curcas* is commonly grown as hedges and fences around gardens and households in Northern and Westerns Nigeria. It is also useful for medicinal purposes in subtropical and semi-arid regions of the world. Previous reports shows that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism [20, 22, 31] as well as having molluscidal and insecticidal properties [25]. *Jatropha curcas* latex has been reported to have strong antimicrobial activities against a wide range bacterial and fungal species but found to be rather unstable leading to loss of activity with time [23].

Carica papaya [family Caricaceae], commonly called pawpaw [English], Ibepe [Yoruba-Nigeria] or Okroegbe [Igbo-Nigeria], is a tree-like herbaceous plant, widely cultivated for its edible fruits in tropical and subtropical regions [7]. It is commonly known for its food and nutritional values throughout the world as well as for therapeutic remedy (14). For example, the fruit contains certain immune-stimulating and anti-oxidant agents, immature fruits and roots are used for their abortifacient activity [3]; the seeds are used as a potential post-testicular antifertility drug; the pulp is used by African hospitals for treating wounds and burns [27]. The latex and the seeds are used in the treatment of gastrointestinal nematode infections and they have shown anthelmintic activity. Also the seeds and immature fruit have shown inhibitory activity against human enteric pathogens [3].

MATERIALS AND METHODS

Sample sources

The samples used for this study were obtained from Akungba-Akoko and the neighbouring community in Ondo State, Nigeria. This includes the water samples form where the aquatic organisms were isolated and the medicinal plants tested for their therapeutic activity.

Sterilization

All glass wares were sterilized in the oven at 170^0C for two hours and allowed to cool down before use. The media used including nutrient agar and sabouraud dextrose agar were also sterilized in an autoclave at 121^0C for 15 minutes.

Procedure for the preparation of the plant extract used
Four different plants extracts used from *Azadirachta indica* [Neem], *Ocimum gratissimum, Carica papaya* and *Jatropha* were prepared separately by washing them in hypochloride solution for the purpose of the surface-sterilizing them. The leaves are then grounded with a clean mortar and pestle and subsequently drain to obtain a sizeable volume of the plant extract which were stored in the refrigerator until when needed. The leaves of the three plants were used in the preparation of the plant extract.

Evaluation of the antifungal activities of plant extracts
The antifungal activities of the plant extracts used against the isolated fungal species were evaluated by the agar well diffusion method as which is a modification of Kirby Bauer, [6] method. This was done by sinking four small wells on a prepared sterile potato dextrose agar with the aid of a sterile cork borrower. The prepared plant extract were then introduced into the holes of different prepared plates into which a 72 hour old inoculums of the isolated fungal species were subsequently separately introduced and incubated at room temperature for 72 hours. The effect of the plant extract on the growth of the isolates were then evaluated based on comparative study with a corresponding control plate which were inoculated with an inoculums of the fungal isolate but without a previous introduction of the plant extract.

RESULTS

This study shows various forms of fungal species isolated from aquatic sources in Akungba-Akoko, Ondo State and some typical medicinal plants that were tested for their antifungal activity on them. Figure 1, shows distribution pattern of the fungal species encountered in the water sample sources. This will help to determine the nature of isolates that are obtainable from different ecological zones. Aquatic fungal isolates from well, slow moving water and fast moving water sources were used for the study.

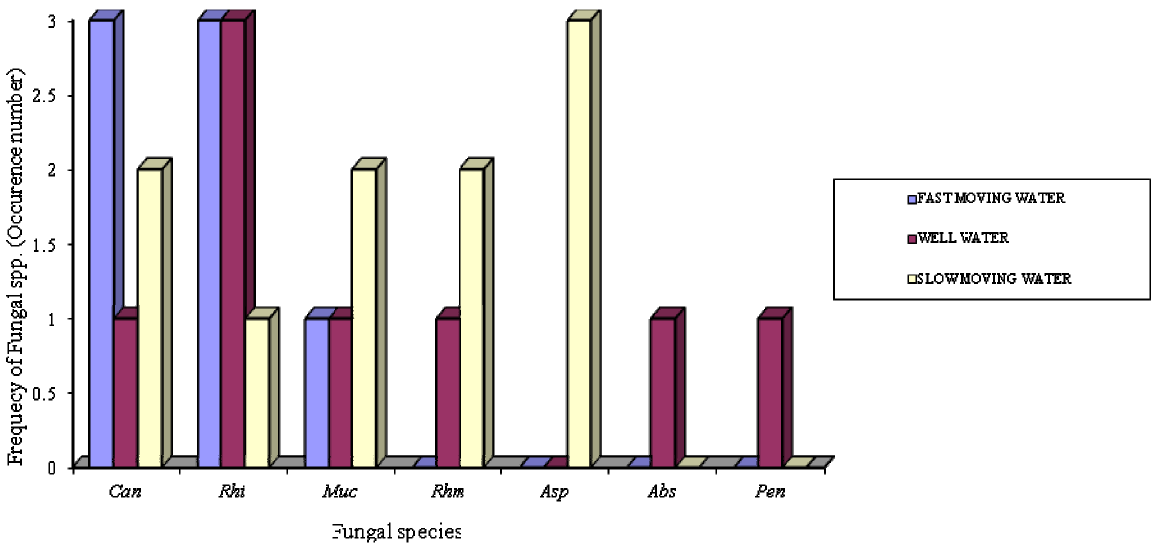

Fig 1: Distribution pattern of fungal species encountered in water sample sources

Candida spp	*Can;*	*Rhizopus* spp	*Rhi*
Aspergillus spp	*Asp;*	*Rhizomucour* spp	*Rhm*
Mucour spp	*Muc;*	*Penicillium* spp	*Pen*
Absidia spp	*Abs*		

Tables 1 and 2 shows the susceptibility pattern of the Fungal species from fast and slow moving water sources respectively to the four plant extract tested while Table 3 show the susceptibilities of the fungal isolates from well water to the medicinal plants. The evaluation of the antimycotic properties of these plant extract determined through the antifungal activity from these sources showed their potency against most of the fungal isolates with different levels of growth inhibition. Out of the four plant sources, extract from *Azadirachta indica* was found to be most active with inhibition zone greater than 15mm and total growth inhibition in some instances against the fungal isolates while *Carica papaya* extracts has lowest activity. This signifies that the active medicinal plant source can be optimized for future antimycotic therapy in diseases control and may solve some antimicrobial resistance problems being from natural origin.

TABLE 1: Susceptibility of the Fungal Isolated from Fast Moving Water Source to the Three Plant Extract Used

ISOLATES	*Azadirachta indica*	*Ocimum gratissimum*	*Carica papaya*	*Jatropha spp*	
FMA A	++	+	+	+	*Candida species*
FMA B	++	+	_	+	*Rhizopus species*
FMA C	++	+	+	+	*Rhizopus species*
FMA D	+	+	_	+	*Mucor species*
FMA E	++	+	+	+	*Candida species*
FMW F	+	+	+	+	*Rhizopus species*
FMW G	+	+	_	+	*Candida species*

+++: Totally prevent fungal growth
++ : Zone of inhibition >15mm -Significantly affect fungal growth
+ : Zone of inhibition –12mm to 15mm -Moderately affect fungal growth
_ : No significant effect on fungal growth

TABLE 2: Susceptibility pattern of the fungal isolates from slow moving water source to the medicinal plant.

ISOLATES	Neem	*Ocimum gratissimum*	Carica papaya	Jatropha	
SMW A	+	+	+	+	*Candida species*
SMW B	++	+	+	+	*Aspergillus species*
SMW C	++	+	+	++	*Rhizomucor species*
SMW D	+	+	+	+	*Rhizopus species*
SMW E	+	+	+	+	*Mucor species*
SMW F	+	+	_	+	*Rhizomucor species*
SMW G	++	+	+	+	*Candida species*
SWW H	++		+	++	*Aspergillus species*
SMW I	++		+	+	*Mucor species*
SMW J	+		_	+	*Aspergillus species*

+++: Totally prevent fungal growth
++ : Zone of inhibition >15mm -Significantly affect fungal growth
+ : Zone of inhibition –12mm to 15mm -Moderately affect fungal growth
_ : No significant effect on fungal growth

TABLE 3: Susceptibility of the Fungal Isolates from Well Water Sources to the plant extracts.

ISOLATES	Neem	*Ocimum gratissimum*	Carica papaya	Jatropha	
WW A	++	+	+	+	*Candida species*
WW B	++	+	+	++	*Rhizopus species*
WW C	+++	+	+	+	*Rhizomucor species*
WW D	+	+	_	+	*Asidia species*
WW E	++	+	+	+	*Rhizopus species*
WW F	+	+	+	++	*Mucor species*
WW G	++	+	++	_	*Rhizopus species*
WW H	++		+	+	*Penicillium species*

+++: Totally prevent fungal growth
++ : Zone of inhibition >15mm -Significantly affect fungal growth
+ : Zone of inhibition –12mm to 15mm -Moderately affect fungal growth
_ : No significant effect on fungal growth

DISCUSSION

The water sources used in the course of the experiment contains different fungal species of diverse nature tested with some medicinal plants for chemotherapeutic purposes. Fungal species isolated from the three water sources include *Candida* species, *Rhizopus* spp., *Rhizomucor* spp, *Mucor* spp, *Aspergillus* spp and *Penicillium* spp. Fungal groups such as *Candida* spp, *Rhizopus* spp. and *Mucor* spp were found to be common to the three water sources sampled. *Rhizomucor* spp was found to be present in water samples from well water and slow moving water sources. *Aspergillus* spp were present in water sample from slow moving water sources while *Absidia* and *Penicillium* species were commonly encountered in water sample obtained from well water sources. This is in corroboration with previous investigation by St-Germine [28] who clarified the presence of typical organisms in contaminated soil or environmental samples including food. Similarly, organisms like Aspergillus species are medically relevant pathogens. Members of the genus are also sources of natural products that can be used in the development of medications to treat human disease [15, 24].

The fungal species isolated from the water sources as linked with previous reports show that they are potentially pathogenic depending on the immune status of an individual using these water sources. This is consistent with the study of Dorothy, [8] and Ahmed, [1] who demonstrated the pathogenic nature of some fungal species from environmental sources. Most of these fungal sources have the ability of growing in variety of synthetic medium studied for their diagnostic purposes [2]. On this basis for chemotherapeutic purposes some medicinal plants tested were *Azadirachta indica*, *Ocimum gratissimum*, *Jatropha* species and *Carica papaya*. The evaluation of the antifungal activities of plant extracts from this sources showed that the medicinal plants exert some antifungal properties against almost all the fungal isolates although at different levels with most of the isolates moderately affected while the growth of only very few of the isolates were totally inhibited by the plants isolates. Out of the three plant extract used, extract from Neem was found to be most active against the fungal isolates while the extract from *Carica papaya* was found to be least active. It can be concluded that the four plants used in this study are potential sources of active antifungal compounds that can be employed for therapeutic and sterilization purposes. The extract with further study can be optimized and purified for effective therapeutic purpose.

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