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Antifungal activity of *Mangifera indica* leaf extracts on selected fungi

DISEGHA, G. C. & AKANI, N.P.
Rivers State University, Port Harcourt, Nigeria

ABSTRACT Antifungal activity of different concentrations of *Mangifera indica* (mango) crude leaf extracts on some selected fungal species, namely, *Candida albicans*, *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus niger*, was studied using standard methods. The extracts prepared were fresh cold aqueous (FCAE), fresh hot aqueous (FHAE) and fresh ethanol extract (FEE) while cold distilled water, hot distilled water and 95% ethanol were used as controls, respectively. Inhibition zones were observed and measured. All fungal isolates were resistant in the controls but showed 100% sensitivity in the standard (Fulconazole, an antifungal). There was a significant ($p \geq 0.05$) increase in the zone of inhibition with increased concentration of the extracts. The fresh hot aqueous extract (FHAE) showed the strongest inhibitory activity with diameter of 18.0±2.0mm against *C. albicans*, 12.1±2.0mm against *A. flavus,* 11.0±1.0mm against *A. niger* and 8.33±0.58mm against *A. fumigatus* at 40mg/ml (40% concentration) while FCAE showed minimal activity. The minimum inhibitory concentration (MIC) of fresh cold and hot aqueous extract of *M. indica* was 20mg/ml for all the test organisms. The standard antifungal agent (Fulconazole), however showed the highest inhibitory activity for *A. niger* (27.0±0.00 – 28.0± 0.00mm). Since the cold distilled water, hot distilled water and 95% ethanol controls did not show any activity on the test organisms, it could be concluded that the antimicrobial activities shown by both fresh cold and fresh hot aqueous extracts was due to the effect of the extracts. The study has revealed that *M. indica* extracts can be used as an antifungal agent in the treatment of infectious diseases implicating the test organisms.

Keywords: Antifungal activity, fungi, minimum inhibitory concentration, *Mangifera indica*

Introduction

Mangifera indica is a species of mango. It belongs to the *Anacardiaceae* family. Its mesocarp is juicy overlaying a seed stone. All mangoes belong to the group of flowering plants and are abundantly found in the Tropics, as well as in other areas of the World (Fayaz, 2011). It is found in the wild in Nigeria and there are several cultivated varieties in farms. It has its secondary or adaptive use for vegetables, dyes, tannins, ornamentals, forages, timbers, auxiliary plant, fuel plant, medicinal plant, essential oils and exudates (Aigbokhan, 2014).

Mango is a well-known and widely used for treatment of several bacterial infections due to its anti-microbial properties (Disegha and Teyor, 2014). The mango is believed to possess antioxidant, antiallergenic, and anti-inflammatory properties (Ajila, *et al.,* 2010). Several reports on antimicrobial activities of mango ex-

tracts or its phytochemicals are available (Hussain, *et al.*, 2010). Phytochemical isolated from mango (*Mangifera indica* L.) leaves, at various concentrations, have been reported to have significant growth suppression on five fungal namely *Alternaria alternata* (Fr.) Keisslers *Aspergillus fumigatus* Fresenius, *Aspergillus niger* van Tieghem, *Macrophomina phaseolina* (Tassi) Goid. and *Penicillium citrii.* (Hussain, *et al*., 2010).

Aspergillus species are implicated in Aspergilloses and in diverse mycoses, especially in man, with disastrous consequences (Cheesebrough, 2010; Mitchell, 2007). The natural abundance of plants in tropical countries such as Nigeria gives an advantage in the use of these natural resources. These have drawn the attention of researchers as well as environmental scientists to be concerned with converting plants to various utilizable forms, especially in therapeutic and prophylactic medicine, nutritional seasons, deodorization and in preventing spoilage of food (Okigbo and Igwe, 2007).

All parts of *Mangifera indica* have therapeutic uses articles (Ribeiro *et al.,* 2007). The powder of tender leaves is used in diarrhoea and diabetes. The smoke by burning of leaves is inhaled for relieving hiccup, catarrhal and throat affections. The ash for leaves is obtained by burning them and it is used as a dusting powder over burns, scalds and other similar complaints. Leaves powder is locally applied to cuts, ulcers and for septic purpose (Kumar, 2002). The leaves are useful for toning up the gums by masticating them. An infusion of leaves and bark is used as an astringent; and is applied as a mouthwash for toothache, sore gums and sore throat and similar affections. The fresh leaf juice of mango is used for treating inflammation of eye; it is applied on eyes twice daily.

The recurring infections associated with fungi cannot be overemphasized. *Aspergillus niger* is implicated in plant pathological conditions (Abbey, 2007) as well as human diseases as opportunistic infections causing ear infections (Schuster *et al*., 2002; Denning *et al.,* 2016). *Aspergillus flavus* known to cause aspergillosis also produces a toxin (aflatoxin) which is one of the aetiological agents for hepatocellular carcinoma (Klich, 2007; Goncalves, *et al.,* 2013). *A. flavus* sometimes causes losses in silkworm hatcheries (Crawford, 2005).

Aspergillus fumigatus is a fungus of the genus *Aspergillus* and is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency (O'Gorman, 2008). In immunocompromised individuals, such as organ transplant recipients and people with AIDS or leukemia, the fungus becomes pathogenic, over-running the host's weakened defenses and causing a range of diseases generally termed aspergillosis which include chronic pulmonary infections (Feldmesser, 2007; Segal, 2009; Ben-Ami *et al.,* 2010).

Recalcitrance of lower fungi to antimicrobial therapy and the consequent threat of to life; increase in the cost, and allergic reactions produced by synthetic drugs; emergence and prevalence of resistant strains of bacteria and fungi due to use and abuse of synthetic drugs, and existence of fake, and non-authenticated drugs and to appreciate the gift of nature to better utilize the resources available in the tropical environment serve as propelling forces for this study.

As noted earlier, *Mangifera indica* is used commonly as a therapeutic agent for various infections and pathological conditions. “Studies indicate that mango possesses antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties” (Shah *et al.,* 2010).). Notable effects such as antibacterial, antifungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypoli-

pidemic, antimicrobial, hepatoprotective, gastroprotective have also been studied"(Shah *et al.,* 2010) with good results. The above justifies the use of *Mangifera indica* in the present context as findings in literature are "very encouraging and indicate that this herb should be studied more extensively to confirm these results." (Shah *et al.,* 2010; Qayyum*, et al.,* 2017).)

The study attempts to investigate *in vitro* antifungal activities on three species of Aspergillus – *A. niger, A. fumigatus A. flavus and Candida albicans* using *Mangifera Indica* leaf extracts. *Mangifera indica.*

Materials and Methods

Collection of Samples

Fresh Mango (*Mangifera indica*) leavers were collected from the Agricultural Farm of the Rivers State University, Port Harcourt, Nigeria, and test organisms - *A. niger, A. fumigatus, A. flavus* were isolated from spoilt food items - bread, gari, and yam purchased from the open market. And pure culture of the experimental *Candida albicans* was obtained from Braithwaite Memorial Specialist Hospital (BMH) in Port Harcourt Rivers State.

Extraction of Plant Material

Preparation of plant samples (Crude Extracts)

Fifty grammes of fresh and dry leaves of *Mangifera indica.* were separately ground using precleaned and oven-dried Corona mechanical grinder (Model 121) to obtain marcerate and powdery samples respectively. 10g, 20g, 30g and 40g of each sample were weighed and mixed with in 90ml, 80ml, 70ml and 60ml of cold distilled water, hot distilled water and 95% ethanol in titration flasks. This mixture yielded 10%, 20%, 30% and 40% (w/v) concentration of crude extracts of Fresh Cold Aqueous Extract (FCAE), Fresh Hot Aqueous Extract (FHAE) and Fresh Ethanolic Extract (FEE). (Disegha and Akani, 2015). The flask was covered with cotton plug and then wrapped with aluminium foil and shaken vigorously (Mustapha, *et al.,* 2014). The crudes were kept for 24 hours and then filtered separately using sterilized Whatman's No. 1 filter paper. The crudes were stored in refrigerator at 4^{o}C until required for the antifungal test (Disegha and Aknai, 2015).

Determination of Minimum Inhibitory Concentration (MIC)

The prepared concentrations that exhibited antifungal activity were incorporated into Sabouraud's broth at 10-fold serial dilution (1st to 4th dilution). To achieve this, 1.0 ml of each extract from the stock concentrations were put into 9.0 ml of sterile Sabourauds broth giving a 1:10 dilution. Subsequent dilutions were made up to 10^{-4}. This was done for all test organisms. 0.1ml suspensions of microorganisms using McFarland standard were incorporated into each test tube after which they were thoroughly shaken and incubated at 37^{0}C for 24 hours. Controls were set as in the screening test. Fulconazole was used as positive control, and solvents for extract

were used as negative controls. Positive control contains 9.0 ml of Sabourauds broth and organism without extract; negative control containing 9.0 ml of Sabourauds broth and extracting solvent with test organism. These were shaken and incubated alongside the inoculated ones. The dilution tubes were then examined for turbidity and tubes showing no turbidity were noted. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of test extract that completely inhibited growth of the test fungi after 48 hours incubation at ambient (25 – 28^{0}C) temperature (Prescott *et al*., 2008).

Determination of Minimum Fungicidal Concentration (MFC)

0.1ml from tubes containing the minimum inhibitory concentrations of the various extracts and the preceding tubes along with the control tubes were sub-cultured on SDA plates and further incubated for 48 hours at ambient (25 – 28^{0}C) temperature . The MFC was determined by observing for failure of spores on their respective plates. Minimum fungicidal concentration (MFC) is the highest dilution or concentration that yielded no single fungal spore (Akinyemi *et al*., 2006).

Results

Result of this study showed various reactions to the treatment with the crude extracts. The test organisms were resistant to treatment with the 10% concentration of the aqueous and fresh ethanolic extract (FEE) at all concentrations applied (Table 4), whereas, all test organisms were susceptible to treatment with the standard antifungal drug Fluconazole (STD).

The fresh cold aqueous and fresh hot aqueous crude extracts of *Mangifera indica* showed a significant ($p \geq 0.05$) increase in the zone of inhibition with increased concentration of the extracts, whereas the fresh ethanol extracts did not show any activity on the test organisms Tables, 2, 3 and 4).

The fresh hot aqueous extract (FHAE) showed the strongest inhibitory activity with zone of inhibition diameter of 18.0±2.0mm against *C. albicans*, 12.1±2.0mm against *A. flavus,* 11.0±1.0mm against *A. niger* and 8.33±0.58mm against *A. fumigatus* at 40mg/ml (Table 3), while fresh cold aqueous extract (FCAE) showed least activity on *A. niger* at 20% concentration (Table 2). Tables 1 to 4 displays the various results of the test performed on the organisms using the specified extract types. In all test organisms, there was increasing zones of inhibition with respect to the concentration.

In the assays with Fresh Cold Aqueous extract (FCAE), the range of effective zone sizes and respective organisms were: 4.33±0.58 to 8.0 ±1.0 (*C. albicans*); 4.0±1.0 to 10.67±1.53 (*A. flavus*); 5.0±1.0 to 8.33±1.5 (*A. fumigatus*), and 3.33±1.16 to 9.33±0.58 (*A. niger*) all corresponding to 20% to 40% concentration of the extracts (Table 2).

In the case of fresh hot aqueous extract (FHAE), the range of effective zone sizes and respective organisms were: 7.0±1.0 to 18.0 ±2.0 (*C. albicans*); 7.67.0±1.16 to 12.0±2.0 (*A. flavus*); 4.00±1.0 to 8.33±0.58 (*A. fumigatus*), and 8.0±1.0 to 11.0±1.0 (*A. niger*) all corresponding to 20% to 40% concentration of the extracts (Table 3)

Table 1: Antimicrobial activity of *Mangifera indica* extracts

Test Organism	Plant Ex-tract	Concentration of Extracts (g/ml)				
		10 %	20%	30%	40%	Fulcona-zole
Candida albi-cans	FCAE	R	S	S	S	S
	FHAE	R	S	S	S	S
	FEE	R	R	R	R	S
Aspergillus niger	FCAE	R	S	S	S	S
	FHAE	R	S	S	S	S
	FEE	R	R	R	R	S
Aspergillus flavus	*FCAE*	R	S	S	S	S
	FHAE	R	S	S	S	S
	FEE	R	R	R	R	S
Aspergillus fumigatus	FCAE	R	S	S	S	S
	FHAE	R	S	S	S	S
	FEE	R	R	R	R	S

Key: S – Sensitivity, R – Resistant, FCAE – Fresh cold aqueous extract, FHAE – Fresh hot aqueous extract, FEE – Fresh ethanol extract

Table 2: Sensitivity pattern (showing mean zones of inhibition in mm) of fungal isolates to different concentrations of Fresh cold aqueous extracts of *Mangifera indica* leaves

Conc. of fresh cold aqueous extract (%)	Zone of Inhibition in mm			
	Candida albicans	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*
10	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
20	4.33±0.58[b]	4.0±1.0[b]	5.0±1.0[b]	3.33±1.16[b]
30	6.0±1.0[c]	7.0±1.0[c]	7.0±1.0[c]	4.67±0.58[c]
40	8.0±1.0[d]	10.67±1.53[d]	8.33±1.5[d]	9.33±0.58[d]
Standard (Fulconazone)	20.0±0.0[e]	28.0±0.0[e]	19.0±0.0[e]	20.0±0.0[d]

*Means with the same superscript along the columns are not significantly different ($p \geq 0.05$)

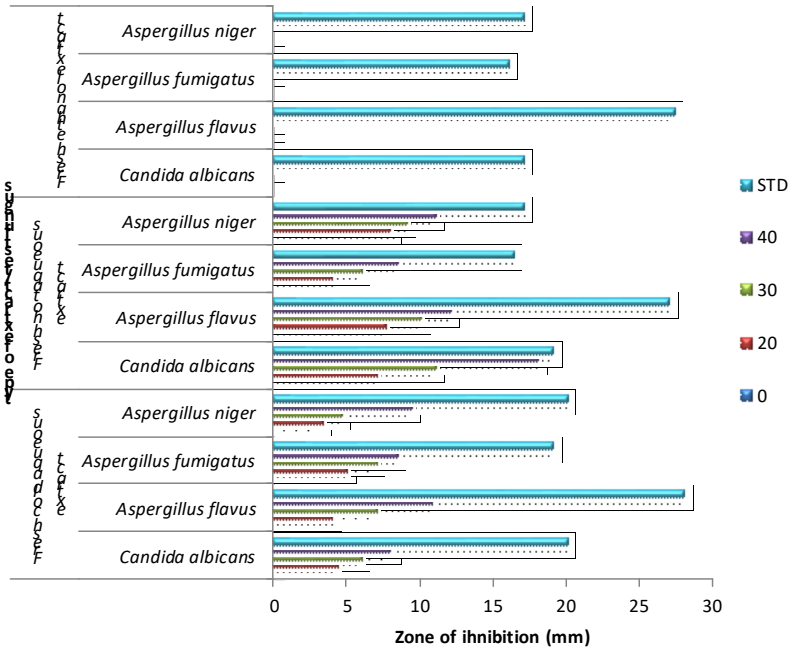
Table 3: Sensitivity pattern (showing mean zones of inhibition in mm) of fungal isolates to different concentrations of Fresh hot aqueous extracts of *Mangifera indica* leaves

Conc. of fresh hot aqueous extract (%)	Zone of Inhibition in mm			
	Candida albicans	*Aspergillus flavus*	*Aspergillus fumigates*	*Aspergillus niger*
10	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
20	7.0±1.0[b]	7.67±1.16[b]	4.0±1.0[b]	8.0±1.0[b]
30	11.0±1.0[c]	10.0±1.0[c]	6.0±1.0[c]	9.0±1.0[b]
40	18.0±2.0[d]	12.0±2.0[c]	8.33±0.58[d]	11.0±1.0[c]
Standard (Fulconazole)	19.0±0.0[d]	27.0±0.0[d]	16.33±0.58[e]	17.0±0.0[d]

*Means with the same superscript along the columns are not significantly different ($p \geq 0.05$)

Table 4: Sensitivity pattern (showing mean zones of inhibition in mm) of fungal isolates to different concentrations of fresh ethanol extracts of *Mangifera indica* leaves

Conc. of Fresh ethanol extract (%)	Zone of Inhibition in mm			
	Candida albicans	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*
10	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
20	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
30	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
40	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
Standard (Fulconazole)	17.0±0.0[b]	27.33±1.16[b]	16.0±0.0[b]	17.0±0.0[b]



Key: STD, Standard (Fulconazole)
Fig. 1: Inhibitory activities of the different extracts of the fungal isolates

Discussion

Result of this study showed that all fungal isolates were resistant in the controls and fresh ethanolic extracts but showed susceptibility in the crude extracts (Table 1). The fresh cold aqueous and fresh hot aqueous crude extracts of *Mangifera indica* showed a significant ($p \geq 0.05$) increase in the zone of inhibition with increased concentration of the extracts whereas the fresh ethanol extracts did not show any activity on the test organisms Tables, 2, 3 and 4). Generally the crude extract had the highest zone of inhibition (Fig.1). The fresh hot aqueous extract (FHAE) showed the strongest inhibitory activity with diameter of 18.0±2.0mm against *C. albicans*, 12.1±2.0mm against *A. flavus,* 11.0±1.0mm against *A. niger* and 8.33±0.58mm against *A. fumigatus* at 40mg/ml (Table 3) while Fresh cold aqueous extract (FCAE) showed least activity on *A. niger* at 20% concentration (Table 2).

The high inhibitory activity of crude extract, especially in the fresh hot aqueous extract (FHAE) is likely due to the presence of triterpenoids, magniferin (xanthine glucoside, isomagniferin) humulene, elemene, ocimene, linalool, nerol, in the plant extract (Ross, 1999). Phytochemicals like flavonoids are known to prevent gastric ulcer due to astringent and antimicrobial properties. These properties may have been inhibited or destroyed when ethanol is used as solvent of extraction. Hence the ethanolic extract were not effective in all assays in this context. Also this difference may probably be that the active ingredients were soluble in water and not in ethanol. Another reason for the low activity of fresh cold aqueous extract could be attributed to the extracts not being prepared according to the traditional method which involved boiling in water for a prolonged time (Disegha and Akani, 2015).

Concentration of 0.32g/ml of Fulconazole was used as the positive controls against the test fungi. Fulconazole, standard antifungal showed a higher antimicrobial activity than the aqueous extracts of *M. indica* but had comparable activity with FHAE on *C. albicans*. The antimicrobial activity of the extract of *M. indica* may be due to the presence of phyochemical compounds as described earlier (Ross, 1999; Hussain, *et al.*, 2010). Phytochemicals like flavonoids are known to prevent gastric ulcer due to astringent and antimicrobial properties.

Various susceptibility patterns and differences were observed in the results.The susceptibility patterns of all test organisms were the same i.e. there was no significant difference among them at $p \geq 0.05$, except for *A. flavus*, which showed the highest zone of inhibition (27.33±1.16) and this was significantly different from all other test organisms..

Among the extracts, 40% concentration showed the highest zone of inhibition and this was significantly different from all other concentrations of fresh hot aqueous extractions ($p \geq 0.05$). This highest zone size was showed on *C. albicans* (18.0±2). The lowest in this group was observed in A. niger (3.33±1.16).

In the assays with fresh cold aqueous extract (FCAE), A. flavus showed the highest susceptibility with zone of inhibition (10.67±1.53) and the lowest was with *A. niger* (3.33±1.16).

Conclusion

Since the controls, cold distilled water, hot distilled water and 95% ethanol controls did not show any activity on the test organisms, it implies that the antimicrobial activities shown by both fresh cold and fresh hot aqueous extracts was due to the effect of active principles in the extracts. The study has revealed that *M. indica* extracts can be used as an antifungal agent in the treatment of infectious diseases implicating the

test organisms. Due to the observation of direct proportionality of increasing zones of inhibition with concentration, it is recommended that higher concentration of crude mango extracts are to be used if therapeutic application of aqueous mango extract are to be used in situations implicating the test organisms used in this context.

In urgent cases were already preparation of crude extract of mango are not available, standard antifungal drugs such as fluconazole is recommended in the therapeutic application against fungal infections implicating the test organisms, since it is relatively inexpensive and readily available. In research laboratory practice where bacterial cultures are the cultures of interest, fluconazole may be used as inhibitory agents to be added to culture media to discourage the growth of unwanted fungi, in order to achieve pure cultures of bacteria.

Since all the controls, and ethanolic extracts did not show any activity on the test organisms, it is concluded that the antimicrobial activities shown by both fresh cold and fresh hot aqueous extracts was due to the effect of the extracts. The study has revealed that *M. indica* extracts can be used as an antifungal agent in the treatment and management of infectious diseases in humans, animals and plants, infected with the test organisms and their related species.

Correspondence
Disegha, G. C.
Department of Microbiology,
Rivers State University, Port Harcourt
Nigeria
Tel.: +234(0)8034739654
Email: gabrieldisegha2@gmail.com

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