Antimycotic and phytochemical screening of the fruit pulp extract of Tamarind (*Tamarindus indica*) on *Candida albicans*

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ABSTRACT

Objective: To determine the antimycotic potency of the aqueous and ethanolic extract of the fruit pulp of *Tamarindus indica* on human pathogenic *Candida albicans.*

Methods: The disk diffusion method was employed to check for the antimycotic potency of extracts while qualitative and quantitative analysis of some phytochemical constituents was carried out following standard methods.

Results: The fruit pulp extracts were effective against the organism at 475 and 485 mg/ml in the aqueous and ethanolic extract respectively. The same concentration served as the Minimum Inhibitory and Minimum Fungicidal Concentration (MIC and MCC) in both extracts. The extracts were also subjected to qualitative and quantitative phytochemical analysis. Alkaloids, tannins and reducing sugars were found in the ethanol extract while in the aqueous extract glycosides, saponins and reducing sugars were discovered to be present.

Conclusions: The study indicated that aqueous and ethanolic extracts of the fruit pulp of *T. indica* can be a potential source of antimycotic agent to combat the challenge of the emergence of drug-resistance in *Candida albicans* and the need to produce more effective antimicrobial agents.

KEY WORDS: Tamarind
Antimycotic
Candida
Phytochemical

Introduction

The last two decades have witnessed a dramatic rise in the incidence of life threatening systemic fungal infections and more importantly the development of resistance to synthetic antifungals by *Candida albicans*. Microbial multiple drug resistance toward commonly used commercial drugs has resulted in an increase in the search for antimicrobial agents from natural sources. The challenge has been to develop effective strategies for the treatment of candidiasis and other fungal diseases, considering the increase in opportunistic fungal infections in human immunodeficiency virus-positive patients and in others who are immuno-compromised due to cancer chemotherapy and the indiscriminate use of antibiotics. The majority of clinically used antifungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of multiple drug resistant strains. Concerns have been raised about both the environmental impact and the potential health risks related to the use of these compounds [1]. Hence, there is a great demand for novel antifungals belonging to a wide range of natural sources, selectively acting on new targets with fewer side effects. Plant derived antimicrobial agents are largely untapped resources with enormous medical potential and much more investigation is needed in this area.

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Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [2]. Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defence against predators and infections. Many of these plant metabolites have been reported to possess enormous biological and pharmacological activities and are used as chemotherapeutic agents or serve as

the starting point in the development of modern chemotherapy. Phytochemicals are plant chemicals that have protective or disease preventive properties on humans. They include alkaloids, glycosides volatile oils, gum, tannins, steroids, saponins, phlobatannin and flavonoids. The plant *Tamarindus indica* L. (Tamarind) also contains some of these constituents that possess important therapeutic properties which can and have been utilized in the treatment of human and other animal diseases worldwide [3-6]. Ingestion of *T. indica* fruit has also been reported to have an additional beneficial effect on the mobilisation of deposited fluoride from bone by enhancing urinary excretion of fluoride [7]. The plant *Tamarindus indica* L. (Tamarind) belongs to the dicotyledonous plants, family Leguminosae, and sub-family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19,327 species [5, 6]. *T. indica* plant is found in the savannah region of Nigeria where it grows wild in backyards, roadsides wastelands. Despite the use of the plant and its fruits for various purposes by the rural people in Nigeria, *T. indica* is yet to be given the focused research attention it deserves [8].

Previous work on the antifungal activity of methanol and hexane extract of the fruit pulp by Adeola *et al*., reported resistance of *Candida albicans*, hence the present study is aimed at the determination of the antimycotic status and phytochemical profile of the aqueous and ethanolic extracts of the pulp on *Candida albicans* [9].

Materials and methods

Collection of plant material

The specimen was collected from Okete village in Moro local Government, Kwara State and was identified as *Tamarindus indica* in Botany Department of Obafemi Awolowo University, Osun State, Nigeria with voucher number 1314.

Aqueous extract of the extracts

The aqueous extract of ripe pods of *Tamarindus indica* was prepared following the procedure suggested by Daniyan and Mohammed with some slight modification [10]. The fruit pulp was mixed with warm distilled water (50 ^{0}C) in the ratio 1:2 and blended in the electric blender. The mixture was shaken on an electric shaker at 200 rpm for 10 minutes, filtered through No 1 Whatman filter paper and evaporated to dryness at ambient temperature. The crude extract obtained was stored at 4 ^{0}C in the refrigerator for further use.

Ethanolic extract of the extracts

The extract was prepared with ethanol according to the procedure described by Doughari [11]. Fifty grams (50 g) of the pulp was extracted with 100 ml of ethanol and kept on a rotary shaker for 12 hours. The extract was filtered and centrifuged at 500 rpm for 5 minutes on a rotary shaker and the supernatant collected. The supernatant was filtered with a No 1 Whatman filter paper and evaporated to dryness. The crude extract obtained was maintained at 4 ^{0}C till further use.

Collection of test organism

Pure culture of *Candida albicans* was obtained from the Mycology Unit of the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. The organism was sub cultured on Potato dextrose agar slant and maintained at 4 ^{0}C in a refrigerator for further use.

Antimycotic sensitivity test

The method of Jadhav *et al*., as described by Rasheed with some slight modification was used. Pure culture of *C. albicans* was grown overnight in peptone water. Sterile potato dextrose agar (PDA) plates were swabbed with 1ml of the pure culture of the organism and allowed to stand for 30 minutes on the sterile bench for absorption to take place. Wells were made in the plates using a sterile cork borer of 5 mm diameter and 0.1ml of the extracts with different concentrations was added into the wells using a sterile micropipette. Various concentrations used were 475, 480, 485, 490 and 495 mg/ml. The plates were allowed to stand for 2 hours at room temperature for diffusion to take place before the plates were incubated at 30 ^{0}C for 72hrs. The zones of inhibition were measured and expressed in millimeters. Respective solvent controls were also run simultaneously [12,13].

Determination of the Minimum Inhibitory and Fungicidal Concentration

Different concentrations (475 to 495 mg/ml) of both aqueous and ethanolic extracts were made in 9 ml of sterile peptone broth in tubes and the absorbance was read in a spectrophotometer at wavelength of 540 nm. The tubes were autoclaved at 121 ^{0}C for 15 minutes. After this, 1ml of *Candida albicans* cultured overnight in peptone broth at 30 ^{0}C for 12 hours was inoculated into the sterile broth. Then the absorbance was read again at the same wavelength. The tubes were incubated at 30 ^{0}C for 24 hours. Finally the absorbance was read again at the same wavelength. The lowest concentrations that recorded the same absorbance before and after incubation were taken as the MIC. The content of the tubes that showed no growth (MIC) were further plated out on sterile plates of PDA and incubated at 30 ^{0}C for 24 hours. The plates were observed for visible growth afterwards and the lowest concentrations that showed no growth were taken as the MCC. A tube containing peptone broth only was seeded with the test fungus to serve as control [11, 13].

Phytochemical Screening

The aqueous and ethanolic extracts of the specimen was screened qualitatively for the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycoside and reducing sugars according to the method described by Akinpelu and Kolawole [14]. Quantitative screening of total alkaloid, saponins and tannins was also carried out following standard methods [15- 17].

Statistical analysis

All data collected after each experiment was subjected to a one- way Analysis of Variance (ANOVA) using the SPSS (1999 version 19) package and the means were calculated using the Duncan's Multiple Range Test of the same software. Means that were different at 0.05 level were considered significant.

Results

Antimycotic test: the organism was sensitive to the aqueous extract at the various concentrations used while it was only sensitive at 485 and 490mg/ml in the ethanolic extract (Figure 1). MIC and MCC: the minimum inhibitory and cidal concentration (MIC and MCC) was found to be 480mg/ml in the aqueous extract and 490mg/ml in the ethanolic extract respectively (Tables 1-4).

Table 1. Minimum Inhibitory Concentration of aqueous extract of *T. indica* pulp against *C. albicans* at 540nm

Concentration of extract in mg/ml	Growth
475mg/ml	Growth
480mg/ml	No growth
485mg/ml	No growth
490mg/ml	No growth

Table 2. Minimum Fungicidal Concentration of aqueous extract of *T. indica* pulp against *C. albicans* at 540nm

Concentration of extract in (mg/ml)	Growth
480mg/ml	No growth
485mg/ml	No growth
490mg/ml	No growth

Table 3. Minimum Inhibitory Concentration of ethanolic extract of *T. indica* pulp against *C. albicans* at 540nm

Concentration of extract in (mg/ml)	Growth
475mg/ml	Growth
480mg/ml	Growth
485mg/ml	Growth
490mg/ml	No growth
495mg/ml	No growth

Table 4. Minimum Fungicidal Concentration ethanolic extract of *T. indica pulp* against *C. albicans* at 540nm

Concentration of extract in (mg/ml)	Growth
490mg/ml	No growth
495mg/ml	No growth

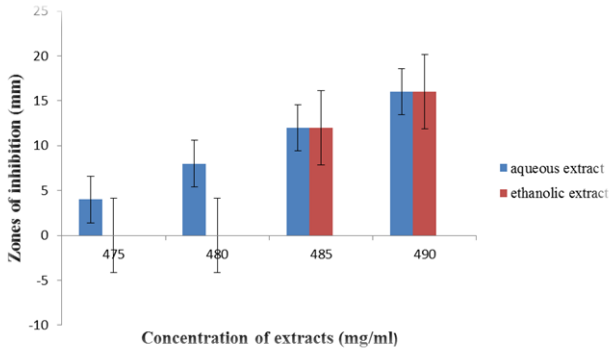
Phytochemical screening: the ethanol extract of the pulp revealed the presence of alkaloids, reducing sugar, tannins and glycosides while the aqueous extract revealed the presence of glycosides and saponin. Quantitatively, alkaloid was found to be high and it was found only in the ethanolic extract while saponin was recorded only in the aqueous extract (Tables 5- 6).

Table 5. Qualitative phytochemical screening of the extracts of *T. indica* pulp

Phytochemical group	Aqueous extract
ethanolic extract	
Alkaloids +	-
Saponins -	+
Reducing sugar +	+
Glycosides +	+
Tannins +	-
Flavonoids -	-

Key: + Positive, - Negative

Figure 1. Antimycotic test of the pulp extracts of *T. indicans* against *C. albicans*



Discussion

The aqueous and ethanolic extract of the fruit pulp of *Tamarindus indica* exhibited antimycotic activity against *Candida albicans*. The antimycotic activity increased as the concentration increased as depicted by the increase in zones of inhibition. The fruit pulp extracts had mycocidal effect on the organism tested. However, the aqueous extract was found to be more effective than the ethanolic extract at similar concentrations (475 and 480 mg/ml). This was contrary to the previous reports that *Candida albicans* was resistant to the hexane and methanol fruit pulp extract of *Tamarindus indica*. This may suggest that the bioactive constituents in the pulp are more active in water and ethanol than methanol and hexane; water and ethanol of course are the two most commonly used extractants at the local level, though the extracts were active at higher concentrations (475-495 mg/ml) than what they initially reported. Earlier authors had reported the antibacterial and antimycotic activities of different parts of the tamarind plant and other plants of the family Leguminosae on various pathogenic bacteria and fungi [11, 13, 18-21]. The two extracts however had similar action on the organism at the highest concentrations used in this study. The mycostatic and mycocidal activities shown by the fruit pulp extract of *T. indica* on *C. albicans* could be attributed to the presence of the phytochemical detected in the plant such as alkaloids, glycosides, saponins and tannins. These secondary plant metabolites have been shown to possess phytoprotective substances with antimicrobial actions that aid in defence mechanism of plant extracts against invading microorganism in humans and animals [22].

In humans, phytochemicals can have complementary and overlapping actions including antioxidant effects, modulations, antibacterial and antiviral effects. Alkaloid was discovered to be the most abundant in the fruit pulp extract of *T. indica* [23]. It was only found in ethanolic extract. Staerk *et al.*, reported that alkaloid was of limited distribution in the plant kingdom but possess antimicrobial, anti-inflammatory and anti-asthmatic properties and is one of the largest groups of phytochemicals in plants with amazing effects on humans [24]. Cardiac glycoside was detected in both aqueous and ethanolic extract. Cardiac glycoside is one of the major phytochemical compounds reported to have been detected in *T. indica* pulp. The traditional use of the pulp in the treatment of heart disease might be attributed to the combinatorial effect of alkaloids, cardiac glycosides and tannins in this plant [25]. The presence of reducing sugar in the aqueous and ethanolic extracts further buttress its immediate assimilation when consumed as food or drug.

The quantitative screening of the ethanol extract revealed substantial amount of alkaloids content (19.46%) higher than 4.32% reported by Abubakar *et* al. [26]. Tannin however, was only detected in the ethanolic extract. The aqueous extract contains saponins in quantities higher than the

2.2% reported by Abubakar *et al*., [26] (Table 6). The different concentration or complete absence of some phytochemicals in either of the extracts is a function of the polarity of the solvent and phytochemicals. This to some extent has effect on the efficacy of the plant extract in treating infections [9]. The effectiveness of the ethanolic extract of the fruit pulp of *T. indicas* in this study could be related to the synergistic effect of the antimicrobial activity of ethanol and the fruit pulp. The susceptibility of the fungus to the aqueous extract at all the concentrations tested is noteworthy and may suggest the reason for the water extraction of the pulp as medicinal agent among the local people.

The presence of these phytochemicals in *T. indica* and its antimycotic activity probably led to the use of the plant (fruit pulp) in treatment of vaginal thrush caused mainly by *C. albicans* in adults by the local people [27].

The plant extracts are potential antimycotic agents against disease and essentially different types of candidiasis infections. The findings in this study revealed that the antimycotic activities showed by the tamarind pulp extracts are likely due to the combine action of the phytochemicals. The investigation has opened up the possibility of the use of this fruit pulp in medical and herbal preparations against infections caused by *Candida.* The phytochemicals detected need to be further studied individually for their mechanisms of action as antimicrobial agents. Consumption of the pulp orally as food and as medicine could be encouraged.

Conflict of Interest

We declare that we have no conflict of interest.

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