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Antifungal Activity of *Parkia Biglobosa* Extract on Pathogenic Strain of *Candida Albicans.*

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ABSTRACT

Medicinal plants play an important role in the development of potent therapeutic agents. Parkiabiglobosais a plant used in traditional medicine for the treatment of certain diseases. In order to scientifically apprise some of the medical uses of parkiabiglobosa, this study aimed at making phytochemical screening and evaluating some antifungal activities of P. biglobosaleave and bark extracts. The major phytochemical constituent of interests such as alkaloids, glycosides and saponins are found to be present in the both aqueous and ethanolic extracts of bark and leaves of Parkiabiglobosa. Aqueous bark extract of Parkiabiglobosaproduced the highest zone of inhibition of (11.60 ± 0.58)at concentration of 50mg/ml which surpassed nystatin (500 IU/ml) with zone of inhibition of (9.33±0.58). The lowest zone of inhibition of 1.03±0.08 was obtained by ethanolic leaf extract at concentration of 2.5mg/ml.The fungitoxicity tests have shown that extracts fromleaf and bark of Parkiabiglobosa at concentration of 50mg/ml can replace synthetic drugs in the management of intertrigoinfection.

Keywords: *Extracts, fungitoxicity, intertrigo, Parkiabiglobosa, medicinal plants,phytochemical.*

INTRODUCTION

In Africa, many species of treesserve as sources of food and for medicinal purposes to indigenous people. *Parkiabiglobosa* tree have been known to be a native of Africa and is an important multipurpose tree of West African Savannah land [1]. Various part of *Parkiabiglobosa* tree are used for medicinal purposes and have high value commercially. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals [2].Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [3]. The use of plants as medicine is recognized as an effective way to discover future medicines.

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [4]. Fungal infections have increased worldwide largely because of the increasing size of people at risk, including immune compromised patients receiving parenteral hyper alimentation and/or broad spectrum antibiotics [5]. Other reasons are increase in immunosuppressive conditions like AIDS and other factors such as organ transplantation, leukemia, diabetes and intravenous drug misuse among others [6]. An important group of the skin pathogens are the fungi, among which dermatophytes and *candida spp*. are prominent [7, 8]. Under certain circumstances usually associated with a compromised host immune system, *Candida albicans* and related species can become pathogenic, causing oral, viginal and/or systemic candidiasis [9]. *Candida albicans*is notorious for causing candidiasis, it can affect esophagus with the potential of becoming systemic, causing a more serious condition

called Candidemia [10]. It can also causes a variety of infections that range from non-life threatening mucosal candidiasis like vaginal yeast infections, thrust, skin and diaper rash to lethal disseminated candidiasis in those with compromised immune system who have an implantable medical device such as peace maker or artificial joint, or who use broad spectrum antibiotcs [11].

Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to those agents [12]. However, since many of the available antifungal drugs have undesirable side effect or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance, some shows ineffectiveness [13] and have become therefore less successful in therapeutic strategies. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. The present investigation is focused on the screening of *Parkiabiglobos a*against human fungal pathogen *Candida albicans.*

MATERIALS AND METHODS

Tested Microorganism

One standard strain of *Candida albicans* was obtained from Microbiology Laboratory of Nigerian Institute for *Trypanosomiasis* Research (Federal ministry of science and technology) Kaduna, Nigeria. These isolates were maintained on Sabouraud dextrose agar SDA (BIOMARK Laboratories, India) at 4^0C. Colonies from the SDA plates were stained by gram staining techniques following the procedure by [14].

Plant Material Collection

Fresh bark and leaves of *Parkiabiglobosa* were collected at Trial Afforestation Research Station, Forestry Research Institute of Nigeria, Afaka Kaduna. The plant parts were chopped and shade-dried at room temperature for 2 weeks then grounded using mortar and pestle to a fine powder in accordance to method described by [15]. The grounded samples were then transported for extraction process.

Preparation of Aqueous and Ethanolic Extracts

The grounded powder was weighed on Satorius balance type (BA 610), 100 g each of the dried samples (bark and leaves) were dissolved in 500ml of 95% ethanol and also 100g of each of the dried samples were dissolved in 1000ml of distilled water separately. After the plant materials were successively extracted with ethanol and distilled water separately, the extract was filtered through (Whatman® No.1, England) in Buchner funnel. This was followed by concentration of the ethanol filtrate on Rotary evaporator type Buchi-R-Switzerland at 50 °C to recover the solvent used and the aqueous filtrate was concentrated using water bath. The filtrate stock solution was kept air dried for further analysis [15].

Phytochemical Screening

The Phytochemical screening procedures carried out were adapted from the previous work on plant analysis by [16, 17] as follows.

Detection of Alkaloids (Wagner's Reagent Test): Extracts were dissolved individually in dilute Hydrochloric acid and filtered and treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Glycosides :(Keller-Killani Test): Five millilitres of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Detection of Flavonoids (Alkaline Reagent Test): Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Detection of Saponins (Foam Test): Each 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Phenols (Ferric Chloride Test): Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of Tannins: Extracts were mixed with 2ml of 2% solution of FeCl3. A blue- green or blue-black coloration indicated the presence of and tannins.

Detection of Steroids: Two millilitres of acetic anhydride was added to 0.5 g of extract with 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Detection of Proteins (Ninhydrin Test): To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Fungitoxicity Test

Different concentration of each plant extract was prepared for studying their antifungal activity following the method described by [18].

Determination of Minimum Inhibitory Concentration (MIC)

The least concentration of the plant extracts that does permit any visible growth of the inoculated test organism in the broth medium was regarded as the MIC in each case. Control experiments were performed without the plant extractsaccording to the method described by [19].

Determination of Minimum Fungicidal Concentration (MFC) of The Extracts

The contents of the tubes that showed no visible fungal growth or turbidity in the minimum inhibitory concentration experiment were cultured into prepared Sabouraud dextrose agar plate to assay for the fungicidal effect of the extracts. The plates containing the test organisms were incubated at 37^0C for 48h. The minimum fungicidal concentration was regarded as the lowest concentration that did not yield any fungal growth on the solid medium used[19].

Result And Discussion

Qualitative Analysis of The Phytochemicals of The Ethanolic And Aqueous Extracts.

Phytochemical screening of the plant extracts showed presence of some phytocompounds (Table 1).Among the phytochemicals determine in this work the phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [20]. They possess biological properties such as anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [21]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [22]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [23]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be anti-microbial substances against wide array of microorganisms' in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [24].

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [25]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [26]. Steroids have been reported to have antibacterial properties [27] and they are very important compounds especially due to their relationship with compounds such as sex hormones [28]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [29].

Table 1.*Qualitative analysis of phytochemicals from plant extracts.*

Phytochemicals ethanolic extracts			aqueous extracts	
	PBL	PBB	PBL	PBB
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	-	+	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroids	-	+	+	+
Phenols	-	+	+	+
Proteins	-	-	+	+

Presence of constituent = +ve Absence of constituent = -ve

***PBL**=Parkiabiglobosa leave, **PBB** =Parkiabiglobosa bark.*

Susceptibility Testing of Aqueous and Ethanolic Plant Extracts and Antifungal Drugs in Culture Media on *Candida Albicans.*

Table 2 shows inhibition zones (mm) of *C. albicans* growth produced by aqueous and ethanolic plant extracts in culture media. Almost all the plant extracts exhibit antifungal effects against *C. albicans*(table 2). In particular, aqueous extracts offer effective bioactive compounds for growth inhibition of *C. albicans*

especially at concentration of 50 mg/ml (Table 2). Even at low concentrations, these plant extracts showed antifungal activity. *Candida albicans* remains the most common infection-causing fungus, about 45% of clinical infections are caused by this pathogen [30]. Despite serious environmental implications associated with the excessive use of chemical fungicides still remains the first line of defense against fungal pathogens. Moreover, these fungicides when ingested by human beings and animals through food and water cause various ailments in the body. Search of natural fungicidal principle, from the plant sources would definitely be a better alternative to these hazardous chemicals.[31, 32, 33] demonstrated that ethanolic extracts of medicinal herbs inhibit growth of *C. albicans*. It was revealed in this study, that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts which also agrees with the report of [34] that higher concentration of antimicrobial substance showed appreciation in growth inhibition.

Table2. *zones of inhibition (mm) of C. albicans growth produced by aqueous and ethanolic leaves and bark extracts of Parkiabiglibosa in culture media.*

Concentration (mg/ml)	Leaf extracts		Bark extract	
	EthanolicAqueous		Ethanolic Aqueous	
2.5	1.03±0.08	2.03±0.58	2.33±1.15	3.67±0.58
5	2.00±0.00	3.17±1.15	3.00±1.15	5.53±1.15
10	2.17±0.58	4.60±1.15	4.33±1.15	7.00±1.00
15	3.37±0.58	6.00±1.00	7.00±0.00	8.00±1.00
20	5.00±0.007	7.03±0.58	8.00±1.00	9.01±0.58
25	6.01±0.00	8.31±0.58	9.00±0.00	9.07±0.58
50	7.00±0.01	9.33±0.58	10.03±0.58	11.60±0.58

Table 3. *Zones of inhibition (mm) of C. albicansgrowth produced by antifungal drugs in culture media.*

Positive and negative control	Inhibition zone (mm)
Nystatin (500 IU / ml)	9.33±0.58
ketoconazole USP 200 mg	6.67±0.57
Distilled water	0.00±0.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) Of Plant Extracts Against *C. Albicans*

The minimum inhibitory concentrations of the plant extracts were presented in table 3.The minimum fungicidal concentration of the extracts proved to possess more fungicidal action against *C. albicans* when they are assayed (table 3).The minimum inhibitory concentration values of the plant extracts against the test organisms showed that fungi vary widely in the degree of their susceptibility to antifungal agents.

This agrees with the report that antimicrobial agents with low activity against an organism have high minimum inhibitory concentration while a highly antimicrobial agent has a low minimum inhibitory concentration [34, 35].

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Plant Extracts Against C. Albicans

Plant parts	Minimum inhibitory concentration [mg/ml]		Minimum fungicidal concentration [mg/ml]	
	Aqueous extracts	Ethanolic extracts	Aqueous extracts	Ethanolic extracts
PBL	0.15	0.31	0.31	0.65
PBB	0.31	0.65	0.65	1.25

PBL*=Parkiabiglobosa leave,* ***PBB****=Parkiabiglobosa bark.*

CONCLUSION

The present study demonstrates the antifungal potentialities of bark and leaves of *Parkiabiglobosa* which would improve our understanding to the biological role of the plant and Future Avenue to develop new pharmacological studies and antifungal therapies.

Bioactive compounds from plants in purified form can replace synthetic drugs and used efficiently against intertigo infection. The results of this study revealed the presence of

medicinally important constituents in the leaf and stem bark of *Parkiabiglobosa*.

Therefore, this plant parts could be seen as a good source of bioactive chemical compounds which can be of great value in drug production. Further work should be carried out to isolate, purify, and characterize the active constituents responsible for inhibiting the growth of *Candida albicans*.

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