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Original Research

Antifungal activities of *Terminalia ivorensis* A. Chev. bark extracts against *Candida albicans* and *Aspergillus fumigatus*

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Summary

Aim: The present study was undertaken to evaluate *in vitro* antifungal activity of aqueous and hydroalcoholic extracts from bark of *Terminalia ivorensis* A. Chev. (Combretaceae).

Methods: *In vitro* antifungal activity of all the extracts was done by agar slant double dilution method. *Candida albicans* and *Aspergillus fumigatus* clinically important strains were used for the study. ketoconazole was used as standards for antifungal assay. Antifungal activity was determined by evaluating of antifungal parameters values which are MCF (minimal concentration fungicide) and IC₅₀ (Concentration for 50% of inhibition) around each assay. **Results:** Result showed that the antifungal activity was more pronounced against *Aspergillus fumigatus* than *Candida albicans*. The hydroalcoholic extract showed best antifungal activity than ketoconazole.

Conclusion: Demonstration of antifungal activity of *T. ivorensis* provides the scientific basis for the use of this plant in the traditional treatment of diseases and may help to discover new chemical classes of antifungal substances that could serve as selective agents for infectious disease chemotherapy.

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INTRODUCTION

Traditional medicine has been practiced for many centuries in many parts of the world, including Africa especially in rural areas to treat their diseases in low cost. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. In spite of the significant number of antifungal for the treatment of the mycosis, many cases of resistance were noted [2]. The development of microorganisms resistance to presently available commercial antifungal has necessitated the search for new antifungal agents. Many studies have been conducted with the extracts of various plants, screening antifungal activity as well as for the discovery of new antifungal compounds [3,4].

The efforts of scientists in establishing plants with promising antifungal property is yielding fruitful results as a number of plants with high antifungal property have been elucidated [5, 6].

Terminalia ivorensis A. Chev. belongs to the family Combretaceae. The plant is used by traditional faith healer to cure many skin troubles, mouth and teeth diseases, cough, diarrheas, diabetes and high blood pressure. The decoction or the bark's powder is used in traditional medicine to look after the wounds, the ulcers, the hemorrhoids, malaria and the yellow fever. The decoction is also used as analgesics in the event against rheumatism and muscular pains. The sheets juice applies to the cuts (wound) and is used inhaling against getting colds. It is also added with barks decoctions in rectal injection against gonorrhoea and

kidney affections. It is also an aphrodisiac. In Côte d'Ivoire, the roots of this plant are used much in the traditional pharmacopeia like toothpick against voices extinctions. The studies carried out in Nigeria showed that the plant is an anti-inflammatory drug and antiarthritic [7]. In Nigeria, in the veterinary medicine, the plant was proved having trypanocides and pesticides properties [8].

In This present work, antifungal activity of *Terminalia ivorensis* bark extracts were investigated against an array of clinically isolated as well as standard microbial cultures.

MATERIAL AND METHODS

Plant Material

The barks of *Terminalia ivorensis* were collected in May, 2006 from the campus site of Nangui-Abrogoua Abidjan, Côte d'Ivoire Western Africa and identified by comparison with specimens : Forest of Adiopodoumé ,Côte d'Ivoire, Western africa, May 17th 1966, Aké-Assi 8855 available at the Herbarium of the floristic national center, Félix Houphouët Boigny University, Abidjan ,Côte d'Ivoire,western Africa.

Microorganisms Studied

In vitro antifungal activity of all the extracts was done by agar slant double dilution method. *Candida albicans* (n° 896/AB du 10.01.2000) and *Aspergillus fumigatus* (n° 896/AB du 10.01.2000) clinically important strains were used for the study, which were both clinical isolates as well as identified strains. ketoconazole was used as standards for antifungal assay. Antifungal activity was determined by evaluating of antifungal parameters values which are MCF (minimal concentration fungicide) and IC₅₀ (Concentration for 50% of inhibition) around each assay. For each extract five replicate trials were conducted against each organism.

Extraction

The barks of *Terminalia ivorensis* were air dried and then powdered in a homogenizer and 100 g was used for different solvent extraction aqueous and hydroalcoholic extracts, the sample was extracted in solvent kept with one liter of water distilled by homogenisation in Blender. After six (6) cycles of homogenisation, homogenate was dried in a fabric square and filtered successively twice with absorbent cotton and once with paper whatman 3mm. Filtrate was concentrated thanks to a Büchi rotary evaporator at 60°C. Dark powder obtained is the aqueous total extract. Hydroalcoholic extract was prepared in the same process by using a mixture of solvent ethanol 70% and water 30%. That gave hydroalcoholic extract.

Antimicrobial Assay

Antifungal tests were carried out on culture medium Sabouraud (BioRAD /Réf:64494 ;Batch:7A2211). Vegetable extract incorporation to agar was made according double dilution method of tilted tubes. 11 test tubes were used including 9 test tubes containing vegetable extract and 2 pilot tubes. Among these two tubes, one without vegetable extract was used as witness of germs control growth while the other without germs and extract was used as witness of culture medium sterility control. Extract concentrations range in the tubes go from 390 to 1.52 µg/mL with geometrical connection of reason ½. All the tubes were pressure-sealed (121°C during 15 min), then tilted with small base at room temperature to allow their cooling and solidification of the agar [9,10]. Germs culture on agar slant previously prepared was made by sowing of 1000 cells of each stock of *C. albicans* and *A. fumigatus* [11, 12]. Cultures were carried out and incubated with 30°C during 48 hours. After this time of incubation, germs were counted with pen of germs meter (CEINCEWARE number 23382) and growth in the 10 experimental tubes was evaluated expressed as survival percentage, calculated compared to 100% of pilot tube survival of growth control. The processing of these data made it possible not only to determine the fungicidal minimal concentrations (FMC), but also to plot the curves of activity of the extracts graphically determine the concentrations for 50% of Inhibition (CI₅₀).

RESULTS

All the barks extracts of *T. ivorensis* were active against *C. albicans* and *A. fumigatus*. However the report/ratio of CMF on *Candida albicans* showed that the hydroalcoholic extract has been 4 times more active than the aqueous extract (Table 1).

Table 1. Antifungal activity of *Terminalia ivorensis* barks extracts against *Candida albicans* and *Aspergillus fumigatus*.

<i>T. ivorensis</i> extracts	<i>Candida albicans</i>		<i>Aspergillus fumigatus</i>	
	MCF (µg/mL)	IC ₅₀ (µg/mL)	MCF (µg/mL)	IC ₅₀ (µg/mL)
Aqueous extracts	390	39.60	195	8.28
Hydroalcoholic extracts	97.5	11.40	97.5	5.16

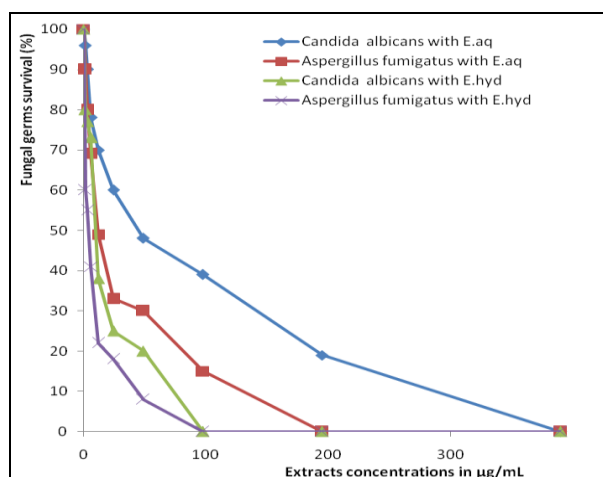


Figure 1. Antifungal of the aqueous extracts and hydroalcoholic activity from *Terminalia ivoriensis* against *Candida albicans* and *Aspergillus fumigatus*

DISCUSSION

Herbal medicine in developing countries is commonly used for the traditional treatment of health problems [13]. In spite of commercial antifungal drugs commonly employed in the treatment of infectious diseases, pathogenic microorganisms keep a resistance [2]. The searching for new antifungal agents is necessitated. In addition to this problem, pharmaceutical drugs (antibiotics) are sometimes associated with adverse effects on host including hypersensitivity, immunosuppression [14]. Therefore there is a need to develop alternative antifungal drugs for the treatment of infections obtained from various sources such as medicinal plants [15,16].

In the present study *T. ivorensis* barks extracted with water and hydroalcohol were investigated for its antifungal potentiality against *C. albicans* and *A. fumigatus* clinically important fungal strains. All the barks extracts of *T. ivorensis* were active against *C. albicans* and *A. fumigatus*. However the report/ratio of CMF on *Candida albicans* showed that the hydroalcoholic extract has been 4 times more active than the aqueous extract. In addition on *Aspergillus fumigatus*, the hydroalcoholic extract was 2 times more active than the aqueous extract. Compared to work of Kporou and al. [17,18] which found a value of $CMF=10^5 \mu\text{g/mL}$ on *C. albicans* with the aqueous extract of *Mitracarpus scaber* a rubiaceae, the aqueous extract of TEKAM 2 in this study is 256 times more active than that of this plant extract. The hydroalcoholic extract of TEKAM 2 is 1,9 times more active than that of *Terminalia catappa* ($CMF = 190 \mu\text{g/mL}$) on *Candida albicans* [19].

The best antifungal activity was shown by the

hydroalcohol extract on both fungal strains with low antifungal parameters values *C. albicans* ($CMF = 97.5 \mu\text{g/mL}$) and *A. fumigatus* ($CMF = 97.5 \mu\text{g/mL}$). The curves of activity with the two extracts against the two fungal strains are on the figure (Figure1). The decreasing shape of the activities curves showed that the 2 extracts have acted according to a relation amount-effect. Compared to the ketoconazole, the extracts tested of *Terminalia* had had a better antifungal activity. The most striking feature of the present findings is that many of the clinical isolates were resistant to the standard pharmaceutical drugs (antibiotics) used while the plant extracts showed moderate to good antifungal activity. The need of the hour is to find new drugs (antibiotics) because the microorganisms are getting resistant to the existing pharmaceutical drugs (antibiotics) [20, 21]. The persistent increase in multi drug resistant strains compels the search for more potent new antifungal. Thus there is a need for a continuous search for new effective and affordable antifungal drugs. The results of present study signified the potentiality of *T. ivorensis* barks as a source of therapeutic agents which may provide leads in the ongoing search for antifungal botanicals.

CONCLUSION

Present study showed that the *T. ivorensis* barks extracts possessed significant *in vitro* antifungal property against 02 clinical isolate as well as identified strains (*Candida albicans* and *Aspergillus fumigatus*). The hydroalcoholic extract exhibited strongest inhibitory effect on (*Candida albicans* and *Aspergillus fumigatus*) as compared to standard antibiotics (Ketoconazole) against the tested microorganisms. Extraction method as described [22,23] concentrated better one or more chemical species against aspergillar and candidosic activity. The present study justified the use in medicine traditional well of *T. ivorensis* to treat skin troubles and as solvents water and the koutoukou (drink extracted from the palm tree traditionally). It is necessary to carry out a bioassay guided fractionation of the extract in a bid to isolate and identify the compounds responsible for the antifungal activity. An elucidation of the mechanisms of action of these extract must be followed by toxicity and *in vivo* tests to determinate the therapeutic applicability of such compounds in combination therapy. These are subjects of ongoing investigation in our research group.

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