Antifungal Activity of the Crude Extracts of *Alafia barteri* Oliver (Apocynaceae) and *Chasmanthera dependens* Hochst. (Menispermaceae)

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The antifungal properties of ethanol and water extracts of the leaves of *Alafia barteri* and the roots of *Chasmanthera dependens* were investigated individually using disc diffusion agar method. The phytochemical properties of the extracts were also assayed. The ethanol water crude extracts of the plants showed definite antifungal activity against *Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Microsporium audonii, Trichoderma viride* and *Trichophyton mentagrophytes*. The ethanol extracts of the plants were more active than their respective water extracts. The extracts had varying zone of inhibition on each of the fungus. The ethanol extracts of *Alafia barteri* had the highest zone of inhibition of 27.42 ± 0.70 mm. The antibiotics, Nystatin, was significantly ($p = 0.01$) more active than the plant extract on most of the fungi, except the ethanol extract of *Alafia barteri* which was insignificantly more active on *Aspergillus niger*, at 10 µg/ml concentration. The crude extracts (ethanol and water) of the two plants contained flavonoid. Tannin was not present in the water extracts of *C. dependens*, while steroid was absent in the ethanol extract of *A. barteri* but present in the other extracts. Anthraquinone, Phylobatanin and Sponin were absent in the plant extracts. The significance of these results was discussed.

Keywords: Antifungal activity; crude extracts; *Alafia barteri; Chasmanthera dependens;* Phytochemical compounds.

Introduction

The use of medicinal plants in the treatment of infection is an age-old practice[1]. The treatment given by the native doctors often includes the administration of entire plants, or extracts of roots, stems, bark, leaves, fruits, seeds or juice of the plant[2]. The treatment might be wrong sometimes, hence the need to scientifically analyse the medicinal plants for their efficacy. In phytopharmacognosy, the schematic search of higher plants for antifungal activity has shown that some plant extracts have the ability to retard fungal growth or completely kill the fungus. Interest in new, safer and more effective antifungal agents has grown with the increasing incidence of fungal resistance especially in immunosuppressive situations[3].

In Nigeria, fungal infections have been treated locally with plants[4]. Some of the medicinal plants used by the Nigerian natives include *Alafia barteri* and *Chasmanthera dependens*. *Alafia barteri* Oliver is in the family Apocynaceae. *A. barteri* is called 'Agbari etu' in Yoruba. It is a high climbing glabrous shrub with pure white or pink flowers in mostly fairly low corymbrose inflorescence, fruiting follicles over 1 m long, slender and faired[5]. *Chasmanthera dependens*

(Hochst) belongs to the family Menispermaceae and its Yoruba name is 'Ato'[6]. *C. dependens* is a shrub and is up to 12 m high[5]. The antifungal and phytochemical properties of *Alafia barteri* and *Chasmanthera dependens* have not been reported in literature.

As a continuation of studies in this laboratory on antifungal components and phytochemical properties of Nigerian medicinal plants[7], antifungal analysis of the crude extract of the leaf of *Alafia barteri* and the roots of *Chasmanthera dependens* are presented here.

Materials and Methods

Source of plant materials

The plant materials, the leaves of *Alafia barteri* and roots of *Chasmanthera dependens* were purchased from Oyingbo market, a native medicinal plant market in Lagos, Nigeria. The plant parts were shade dried at room temperature (28-31°C) for 14 days. Samples of the plants were authenticated by Dr. O.T. Ogundipe of the Department of Botany and Microbiology, University of Lagos, Nigeria, as well as using the text in vernacular names of medicinal plants by Gbile[6]. Voucher samples (LUH 200074 and LUH 200075) have been deposited at the Department of Botany and Microbiology, University of Lagos, Nigeria.

Source of micro-organisms

The fungi used in this work were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Trichoderma viride, Trichophyton mentagrophytes* and *Microsporium audonii.* These micro-organisms were obtained from infected skin scrapings of some patients at the Primary Health Center, Yaba Lagos. These fungi were stored on Sabouraud dextrose agar (oxoid) slants in the refrigerator at 4°C prior to use.

Preparation of soluble extract

The dried leaves of *Alafia barteri* and dried roots of *Chasmanthera dependens* were ground into fine powder with an electric blender respectively. Two 600 g portions of the powdered leaves and two 600 g portions of the powdered roots were soaked separately in 1.8 litres of 70% aqueous ethanol, 1.8 litres of distilled water respectively for 24 hours. Thereafter each extract was filtered through Whatman filter paper No. 1823 and concentrated by evaporating in a rotatory evaporator at 40°C, producing the ethanol and water extracts of each plant part. The two extracts of each plant were stored in the refrigerator at 4°C prior to use.

Screening for antifungal activity

The antifungal activity testing was carried out using the disc agar diffusion method of Irobi and Daramola[8]. Spore or conidia suspensions of 10^5 and 10^7 cells of the fungi, counted with haemocytometre, were made. About 10 ml of previously prepared Sabouraud dextrose agar (oxoid) were poured into petri dishes (9 mm diameter) and allowed to solidify. A micropipette was used to introduce 0.1 ml of the spore or conidia suspensions onto the agar plate, and spread with a glass spreading rod under aseptic conditions. Sterilised discs (6 mm diameter, Whatman No. AA2017006) were soaked in each of the extracts (100 µg/ml) being assayed for 6 hours). Four of these soaked discs were spread on a fungal spore or conidia seeded plate with the help of sterile forceps, three plates were prepared for each fungus per extract. There were two controls: one contained the fungal inoculum but with discs that were soaked in sterile distilled water. The second type of control had the discs soaked in orthodox antibiotics, Nystatin, (10 µg/ml). All the plates containing the discs were then incubated at 28°C-31°C. Zone of inhibition was measured after 72 hours of incubation.

A concentration gradient of minimum inhibition concentration (MIC) of the antifungal extracts was determined by varying the concentration of reconstituted extract (0.01-1000 µg/ml). The antifungal activity testing results were statistically analysed as described by Parker[9].

Preliminary phytochemical studies

Preliminary phytochemical studies were carried out using the methods of Fadeyi *et al.*[10] and Harbone[11]. The ethanol and water extracts of *Alafia barteria* and *Chasmanthera dependens* were screened for the presence of anthocyanin, anthraquinone, butacyanins, flavonoids, phylobutanin, saponin, steroid and tannin.

Results

The powdered *Alafia barteria* leaves produced

TABLE 1

Antifungal Activity of Ethanol and Water Extracts of *Alafia barteri* and *Chasmanthera dependens*

Extracts of solution	Zone of inhibition (mean ± S.Emm)						
	Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Candida albicans*	*Microsporum audonii*	*Trichoderma viride*	*Trichophyton menta-grophytes*
Control (Distilled water)	0.00 ± 0.00a*	0.00± 0.00a	0.00 ± 0.00a	0.00± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Nystatin	25.56 ± 1.02c	26.38 ± 0.81c	15.56 ± 0.44n	26.44 ± 0.78c	31.08 ± 0.40b	28.41 ± 1.75c	32.13 ± 0.77b
Alafia barteri (Ethanol extracts)	14.63 ± 1.04d	0.00 ± 0.00a	16.06 ± 062n	14.00 ± 0.69d	0.00 ± 0.00a	27.42 ± 0.70c	20.06 ± 1.09p
Alafia barteri (Water extracts)	14.19 ± 0.31d	13.25 ± 0.41d	0.00 ± 0.00a	16.50 ± 0.85n	19.55 ± 0.85pk	16.10 ± 0.15n	16.75 ± 0.21n
Chasmanthera dependens (Ethanol extracts)	18.13 ± 0.86k	15.06 ± 0.65n	12.43 ± 0.37ef	11.00 ± 039hf	14.19 ± 0.82d	11.06 ± 0.22fh	25.06 ± 0.95c
Chasmanthera dependens (Water extracts)	0.00 ± 0.00a	11.75 ± 0.28h	0.00 ± 0.00a	10.50 ± 057hg	9.25 ± 0.25g	0.00 ± 0.00a	0.00 ± 0.00a

* Samples with similar alphabet show no significance at p = 0.01.
Samples with different alphabets show significant difference at p = 0.01.

TABLE 2

Phytochemical Compounds Present in the Crude Extracts of *Alafia barteri* and *Chasmanthera dependens*

Medicinal Plant	Phytochemical compounds							
	Anthocyanin	Anthraquinone	Butacyanin	Flavonoid	Phylobutannin	Saponin	Steroid	Tannin
Alafia barteri Ethanol extract	+	–	+	+	--	–	–	+
Alafia barteri Water extract	–	–	–	+	–	–	+	+
Chasmanthera dependens Ethanol extract	+	–	+	+	–	–	+	+
Chasmanthera dependens Water extract	–	–	+	+	–	–	+	–

(+) = Presence of Phytochemical compound.
(–) = Absence of Phytochemical compound.

26 g of ethanol extract and 58 g of water extract. The powdered roots of *Chasmanthera dependens* produced 18 g of ethanol extract and 44 g of water extract. The result in Table 1 shows that all the crude extracts had significant antifungal activity on most of the fungi. The zone of inhibition varied for the fungi with respect to the type of plant extract. The ethanol extract of the plants had a higher zone of inhibition on the fungi than the corresponding water extract. Some extracts did not inhibit the growth of fungi such as the ethanol extract of *A. barteri* on *Aspergillus fumigatus* and *Microsporum audonii,* the water extract of *A. barteri* on *Aspergillus Flavus;* and the water extracts of *Chasmanthera dependens* did not inhibit the growth of *Aspergillus flavus, Aspergillus niger, Trichoderma viride* and *Trichophyton mentagrophytes.* Among the plant extracts, the ethanol extract of *Alafia barteri* had the highest zone of inhibition of 27.42 ± 0.70 mm. The zone of inhibition for the antifungal active extracts were above 10 mm. The antibiotic, Nystatin, was significantly more active than the plant extracts on more fungi except the ethanol extract *Alafia barteri* which was insignificantly ($p = 0.01$) more active (Table 1). The fungi responded to increase in concentration gradient for the antifungal active plant extracts and antibiotics, i.e. zone of inhibition on the fungi increased as the concentration of the antifungal active plant extracts or Nystatin increased.

The crude extracts (ethanol and water) of the two plants contained flavonoid (Table 2). Tannin was not present in the water extracts of *Chasmanthera dependens,* while steroid was also absent in the ethanol extract of *Alafia barteri* but present in the other extracts. Anthocyanin was only present in all the ethanol extracts of the plant parts tested. Anthraquinone, phylobatanin and saponin were absent in the plant extracts.

Discussion

This investigation reveals that the ethanol extracts of *Alafia barteri* and *Chasmanthera dependens* can be effective antibiotics since they inhibited the growth of fungal causal agents of skin disease. This observation is in line with the work of Ajaiyeoba *et al*[4] on *Ritchiea capparoides* var. *longipedicallata.* The ethanol and water extracts had different degrees of antifungal activity. It is similar to the work of Guindidza and Gaza[12] who worked on citric acid, dichloromethane and petroleum ether extract of the bark of the *Dalbergia melanoxylon.* They found that each extract had varying antifungal activity and suggested that the bioactive substance might be more soluble in most of the active extract or fraction.

The presence of biologically active compounds such as flavonoids might have been responsible for the antifungal activity observed in this present study. Flavonoids were implicated by Barnabas and Nagarajan[13] to be responsible for the antifungal activity of some other medicinal plants. It is noted that the crude extracts of the two plants might be host specific in its antifungal activity, due to the varying zone of inhibition the plants have on the fungi.

This current experiment therefore provides some scientific justification for the utilization of extracts from these plants, *Alafia barteri* and *Chasmanthera dependens,* by the Nigerian native to treat skin disease. However, it is important to point out that crude extracts such as these need to be further purified through antifungal activity guided fractionation.

The toxicity level of the pure compounds can be investigated with the view of formulating them into crude drugs of the therapeutic threshold that is acceptable.

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REFERENCES

1. Dalziel, J.M., (1937). *Useful Plants of West Tropical Africa,* Crown agents for the colonies, London, 612 pp.
2. Sofowora, A.E., (1993). *Medicinal Plants and Traditional Medicine in Africa,* Spectrum Books Ltd., Ibadan, 288 pp.
3. Wagner, H. and Bladt, S., (1996). *Plant Drug Analysis,* 2nd edition, Springer, Berlin, 320 pp.
4. Ajaiyeoba, E.O., Rahman, A.U. and Choudhary, I.M., (1998). Preliminary antifungal and cytotoxicity studies of extracts of *Richiea capparoides* var. *longipedicellata, Journal of Ethnopharmacology, 62,* pp. 243-246.
5. Hutchinson, J. and Dalziel, J.M., (1954). *Flora of West Africa,* 2nd edition, (revised by Keay, R.W.J), Crown agents for overseas Governments and Administration, London, 295 pp.
6. Gbile, Z.O., (1984). *Vernacular Names of Nigerian Plants in Yoruba,* Forest Research Institute of Nigeria, 85 pp.

7. Adekunle, A.A., (2001). Ethnobotanical studies of some medicinal plants from Lagos State, Nigeria, *Nigeria Journal of Botany, 14,* pp. 71-79.
8. Irobi, O.N. and Daramola, S.O., (1993). Antifungal activities of crude extracts of *Mitrocarpus villosus* (Rubiaceae), *Journal of Ethnopharmacology, 40,* pp. 137-140.
9. Parker, R.E., (1979). *Introductory Statistics for Biology,* 2nd edition, Edward Arnold, London, 112 pp.
10. Fadeyi, M.O., Adeoye, A.O. and Olowokudejo, J.D., (1987). Epidermal and phytochemical studies in the genus *Bowehavia* (Nyctaginaceae), *Journal of Crude Drug Research, 27,* pp. 178-184.
11. Harbone, J.B., (1998). *Phytochemical Methods* — A *guide to modern techniques of plant analysis* (3rd edition), Chapman and Hall, London, 302 pp.
12. Gundidza, M. and Gaza, N., (1993). Antimicrobial activity of *Dalberigia melnoxylon* extracts, *Journal of Ethnopharmacology, 40,* pp. 121-130.
13. Barnabas, C.G. and Nagarajan, S., (1988). Antimicrobia activity of flavonoids of some medicinal plants, *Fitoterapia 3,* pp. 508-510.