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Research

Antifungal activity of bark extract of *Ficus vallis-choudae* Delile-holl (Moraceae) and *Detarium microcarpum* Guill-Perr. (Caesalpinaceae)

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Abstract

Ethanol and water crude extracts of Ficus vallis–choudae and Detarium microcarpum were investigated individually for antifungal activity by disc diffusion agar method. The phytochemical properties of the extracts were also assayed. The plant ethanol crude extracts had significant (P<0.01) antifungal activity with varying zones of inhibition on Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Microsporum audouinii, Trichoderma viride and Trichophyton mentagrophytes isolated from patients with skin diseases. The ethanol extracts were more active than the corresponding water extract. The ethanol extract of Ficus vallis–choudae caused the highest zone of inhibition (20.8±1.8mm) on Trichoderma viride. The crude extracts of the two plants contained anthocycanin, flavonoids, steroid and tannin.

Keywords: Antifungal activity, tannin, flavonoid, *Ficus vallis-choudae, Detarium microcarpon*

Introduction

The use of medicinal plants in the treatment of infection is an age-old practice (Dalziel, 1937). The treatment given by the traditional medicine practitioners often include the administration of entire plant or roots, stem, bark, leaves, fruits, seeds or juice from local plants sometimes inappropriately. Medicinal plants are sold in large quantities and varieties in local markets to people in search of cures for particular ailments or as usually claimed, all kinds of diseases (Sofowora, 1993). Hence, the need to analyse the plants for their potency and efficacy.

For many years now, interest in new, safer and more effective antifungal agents has grown with the increasing incidence of fungal infections and resistance to antifungal agents (Wagner & Bladt, 1996). In Nigeria, fungal infections have been treated locally with plants. Several studies on antifungal activities of medicinal plants have been conducted by a number of investigators such as Irobi and Daramola (1993) on *Mitracarpus villosum*, Alade and Irobi (1993) on *Acalypha wilkesiana*, Swets and Zeitlinger (1995) on *Cassia alata*, Saxena and Mathela (1996) on *Nepeta leucophylla*, Ajaiyeoba *et al*. (1998) on *Ritchiea capparoides* and Adekunle (2000) on *Brachystegia eurycoma* and *Richardia brasiliensis*.

Ficus vallis-choudae Delile-Holl (Moraceae) and *Detarium microcarpum* Guill-Perr. (Caesalpinaceae) are among the plants listed in Nigeria to cure skin diseases of fungal origin. *F. vallis–choudae* called 'oguro' in Yoruba language (Gbile, 1984) is a forest tree c. 60m high, low branching with a spreading crown (Hutchinson & Dalziel, 1954). An infusion of the bark is used as a wash for leprous ulcers, and the juice of the fruit is squeezed on jigger sores (Berg, 1990). The leaves and young stem are decocted and taken for jaundice, nausea, bronchial and gastro-intestinal troubles (Oliver, 1960). The bark is used as poison antidotes in Ghana and Zaire (Abbiw, 1990). The ash of the wood is also used to make local soap (Burkill, 1997).

Detarium microcarpum known in Yoruba as 'arira' is a large forest tree c. 18m high and 4m in girth with dark brown bark above and orange brown

beneath (Hutchison & Dalziel, 1954). The bark is used to treat haemorrhoids and blennorrhoea in Senegal while it is used for wound dressing and dysentery in Nigeria (Oliver, 1960). The roots are aromatic, scented when heated and used as perfume in Sudan. The plant is deemed medically useful and entered into traditional pharmacopoeas for treating numerous ailments such as diarrhoea, dysenterie, haemorrhoid, leprosy, syphilis, blennorrhoea, rheumatism, impotence, sterility, fungal infection and biliousness (Kerharo & Adam, 1962). Glycosides and alkaloids are present in the leaves of *D. microcarpum* (Iwu, 1986). The antifungal activity and phytochemical properties of the species and those of *F. vallis-choudae* have not been investigated.

In continuation of studies on antifungal components and phytochemical properties of Nigerian medicinal plants in our laboratory (Adekunle, 2001; Adekunle *et al.*, 2003), crude extracts of the barks of *F. vallis-choudae* and *D. microcarpum* were investigated.

Material and methods

Sources of plant materials

The barks of *F. vallis-choudae* and *D. microcarpum* were purchased from Oyingbo market, a native medicinal plant market in Lagos, Nigeria. The plant parts were shade dried at room temperature (28-30^{0}C) for 14 days. Plant samples were identified at the Lagos University Herbarium (LUH) and as described by Gbile (1984). Voucher specimens (LUH 200072 & 200073) were deposited at LUH.

Sources of microorganisms

The fungi (*Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Trichophyton mentagrophytes, Trichoderma viride* and *Microsporum audouinii)* used in this study were obtained from infected skin scrapings of patients at the Primary Health Centre, Yaba, Lagos. The fungal cultures were stored on Sabouraud dextrose agar (Oxoid) slants at 4^{0}C in a refrigerator prior to use.

Extraction:

The dried barks of *F. vallis–choudae* and *D. microcarpum* were ground to a 60-mesh-diameter powder using pestle and mortar, as well as an electric blender (Nakai Japan 462). 600g of each ground plant part was extracted in 1.2 litres of 70% aqueous ethanol and distilled water for 24 hours. Each plant extract was filtered (Whatman filter paper No. 1823) and concentrated by evaporating in a rotatory evaporator (Bibby Sterilin RE 20501) at 40^{0}C, producing the ethanol and water extracts of each plant. The extracts of each plant were stored at 4^{0}C in a refrigerator.

Antifungal testing

Antifungal activity was assessed using the disc agar diffusion method of Irobi and Daramola (1993). Spore or conidia suspension of 10^5-10^7 CFU of the seven fungi, counted with haemocytometer were made. 10cm^3 of prepared Sabouraud dextrose agar (Oxoid) was poured into Petri dishes and allowed to solidify. 0.1cm^3 of the spore/conidia suspensions was added onto the agar plate, and spread under aseptic conditions. Sterilised discs (6mm diameter, Whatman No. AA2017006) were soaked in each of the extracts (100μg/cm^3) for 6 hours. Four of these soaked discs were spread on a fungal spore or conidia seeded plate. Three plates were prepared for each fungus per plant extract. There were two controls. One contained fungal inocula with discs soaked in sterile distilled water. The second had the discs soaked in orthodox antibiotics (Nystatin 10μg/cm^3). All the plates containing the discs were incubated at 28-31^{0}C. Zone of inhibition was measured after 72h incubation.

A concentration gradient or minimum inhibition concentration (MIC) of the antifungal extracts was determined by varying the concentration of reconstituted extract solution (0.01–1000μg/cm^3). The antifungal activity testing results were statistically analysed as described by Parker (1979).

Preliminary phytochemical studies:

Preliminary phytochemical studies were carried out as described by Fadeyi *et al*. (1987) and Harbone (1998). The ethanol and water crude extracts of *F. vallis-choudae* and *D. microcarpum* were screened for the presence of anthocyanin, anthraquinone, butacyanins, flavonoids, phylobatanin, saponin, steroid and tannin.

Results

The powdered bark of *Ficus vallis–choudae* produced 34g of ethanol and 89g of water extracts. The powdered *Detarium microcarpum* bark produced 27g of ethanol and 78g of water extracts. The results of the antifungal screening of the crude extracts of *F. vallis-choudae* and *D. microcarpum* are presented in Table 1. The extracts showed definite significant antifungal activity on most of the fungi. The ethanol extracts were more potent than the corresponding water extracts. The zone of inhibition varied for the fungi tested with respect to the type of extract. Ethanol extract of *F. vallis-choudae* was the most active with 20.8±1.8mm zone of inhibition on *Trichodemia viride*. The water extract of *F. vallis-choudae* was only active on *Trichoderma viride* and *Trichophyton mentagrophytes* while that of *D. microcarpum* was active on *Microsporum audonii* and *Trichodema viride*. The water extracts of the two

TABLE 1:Antifungal activity of ethanol and waterbark extracts of Ficus vallis-choudae and Detarium microcarpum

Fungi/Extract or Solution	Zones of inhibition (Mean ± SE mm)						
	Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Candida albicans*	*Microsporum audonii*	*Trichoderma viride*	*Trictrophyton mentagrophytes*
Control (W)	0.0 ± 0.0a*	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Nystatin	25.6±1.0d*	26.4 ± 0.8d	15.6 ± 0.4b	26.4±0.8d	31.1 ± 0.5e	23.4± 0.7d	32.1 ± 0.8e
F. vallis-choudae (E)	16.8±0.31b	16.8±0.75b	18.5±0.5cl	17.4±1.4b	19.1±0.6kl	20.8±1.8k	17.2± 0.9bc
F. vallis-choudae (W)	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	10.0 ± 0.1f	12.25 ± 0.3fh
D. microcarpum (E)	16.4±0.8b	20.8 ± 1.5k	17.9±0.6cb	15.6±1.2b	15.1 ± 0.8b	17.2±0.7bc	20.69 ± 1.1k
D. microcarpum (W)	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	11.0 ± 0.5f	13.1±0.65h	0.0 ± 0.0a

*Similar alphabet: not significant Different alphabets: significant (P<0.01) W: Water E: Ethanol

species did not show any inhibition against the *Aspergillus* species tested. The antibiotic, Nystatin, had a significant higher zone of inhibition than any of the plant extracts. Nystatin produced the largest inhibition against *Trichophyton mentagrophytes* (32.1mm). The fungi responded to increase in conc-entration gradient for the antifungal active plant extracts and antibiotics. Thus, the zone of inhibition on the fungi increased as the concentration of the antifungal active plant extracts or Nystatin increased.

The crude ethanol and water extracts of the two plants contained anthocyanin, flavonoids, steroid and tannin. Antraquinone, butacyanin, phylobatanin, and saponin were absent from the extract of *F. vallis-choudae* and *D. microcarpum* (Table 2).

Discussion

The significant antifungal action of ethanolic extracts of *Ficus vallis–choudae* and *Detarium microcarpum* (P<0.01) suggests the presence of potent antinfungal components. Ajaiyeoba *et al.* (1998) obtained similar results on *Ritchiea capparoides* var. *longipedicallata*. The presence of biologically active constituents such as flavonoids and tannins might be responsible for the antifungal activity observed in the present study. Inhibition of cell wall formation resulting in death of fungi was attributed to tannins in *Terminalia citrina* extracts (Burapadja & Bunchoo, 1995). Barnabas and Nagarajin (1988) suspected flavonoids to be responsible for the antifungal activity of some other medicinal plants.

The crude extracts of the two plants might be host specific due to the varying zones of inhibition the plants exerted on the fungi.

The antifungal properties of the ethanol and water crude extracts of *F. vallis-choudae* and *D. microcarpum* support the utilization of these forest plants for skin diseases by traditional doctors. However, it is important that the crude extracts be purified further through antifungal activity graded fractionation to pinpoint the active constituents and correct dosage.

TABLE 2: Phytochemical compounds in bark extracts of Ficus vallis-choudae and Detarium micro-carpum

Phytochemical/ Plant extract	Anthoc-yanin	Anthraq-uinone	Butacy-anin	Flavon-oids	Phylob-atanin	Saponin	Steroid	Tannin
F. vallis-choudae (Ethanol)	+	-	-	+	-	-	+	+
F. vallis-choudae (Water)	+	-	-	+	-	-	+	+
D. microcarpum (Ethanol)	+	-	-	+	-	-	+	+
D. microcarpum (Water)	+	-	-	+	-	-	+	+

+ : Present - : Absent

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