

IN VITRO ANTIFUNGAL ACTIVITY OF *DETERIUM MICROCARPUM*

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ABSTRACT

Objective: To evaluate the antifungal activity of root extracts of *Deterium microcarpum* against some pathogenic fungi.

Methodology: Soxhlet extraction of the active principles of the plant was carried out using petroleum ether, methanol and 70% methanol in water and tested for antifungal activity against some pathogenic fungi namely; *Trichophyton mentagrophytes*, *Aspergillus niger*, *Penicillium digitatum*, *Fusarium moniliforme*, *Candida albicans* and *Cryptococcus neoformans* at varying concentrations of 100, 200, 500, 1000, and 2000 µg/ml using the filter paper disc diffusion method. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) was also determined at extract concentration range of 65-1000 µg/ml at room temperature and 37 °C for *C. albicans*.

Results: The extracts were active against all the fungi tested at all concentrations of the extract used with *Candida albicans* showing the least susceptibility. The MIC and MFC values for the extracts ranged between 50-1000 µg/ml.

Conclusion: Antifungal substances can be sourced from *Deterium microcarpum* for possible development of antifungal drugs for the treatment of fungal infections such as candidiasis, cryptococcosis and skin infections.

KEY WORDS: *Deterium microcarpum*, Antifungal activity, Minimum inhibitory concentration, Minimum fungicidal concentration.

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INTRODUCTION

The study of resistance to antifungal agents has lagged behind that of antibacterial resistance.¹ Mycotic infectious rate increased significantly in association with several changes in medical practice, including more widespread

use of therapies that depress the immune system, the frequent and often indiscriminate use of broad-spectrum antibacterial agents, the common use of indwelling intravenous devices, and the advent of chronic immunosuppressive viral infections such as AIDS.² For nearly 30 years, amphotericin B, which is known to cause significant nephrotoxicity, was the sole drug available to control serious fungal infections. The approval of the imidazoles and the triazoles in late 1980s and early 1990s were major advances in the ability to safely and effectively treat local and systemic fungal infections. The high safety profile of triazoles, in particular fluconazole, has led to their extensive usage. Fluconazole has been used to treat in excess of 16 million patients, including over 300,000 AIDS patients, in the United States alone since the launch of this drug.¹ Concomitant with this widespread use, there have been

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increasing reports of antifungal resistance.³ These developments and the associated increase in fungal infections⁴ intensified the search for new, safer, and more efficacious agents to combat serious fungal infections. *Deterium microcarpum* (Caesalpiniodeae Subfamily Leguminosae) is a plant with dense rounded crown shaped leaves and disc-shaped fruits (4cm diameter) with a stem covered with a brown brittle skin that grows up to 10-25m high. The seed is enclosed within an edible sweet greenish coloured pulp mixed with tangle fibre. The Nyandang and Lunguda people of Adamawa State, Nigeria call it *gwogwo* and *takalaka* respectively, while the Hausa (Northern Nigeria) call it *taura*. It is commonly used in the treatment of skin and scalp infections, and diarrhea associated with 'greenish' stooling in children. However, despite the local usage of *Deterium microcarpum* as a medicinal plant, there is a dearth of published medicinal research information on the plant which informs our choice of the plant for evaluation of the antifungal activity.

METHODOLOGY

Laboratory grades of Petroleum ether (BDH) methanol (BDH) and acetone (BDH), and potato dextrose agar (PDA) (Difco; Detroit, Michigan) used for this work were obtained from the Microbiology Laboratory, Federal University of Technology, Yola, Nigeria.

The plant material was collected from the outskirts of the Federal University of Technology Yola, Nigeria and identified by Mr D.F. Jatau of the Department of Forestry and Wildlife Management, School of Agriculture and Agricultural Technology, Federal University of Technology Yola, Nigeria. The roots were chopped into pieces using clean sterile knife, shade-dried (30-32°C), coarsely grounded using pestle and mortar and then reduced to powder using an electric blender (National, MX-795 N) and then stored in well stoppered bottles until use.

The Microorganisms used included standardized fungal strains of *Aspergillus niger*, *Trichophyton mentagrophytes* obtained from the

Microbiology Laboratory of the National Veterinary Research Institute (NVRI) Vom, Nigeria, clinical isolates of *Penicillium digitatum*, *Fusarium oxysporum*, obtained from the Microbiology Laboratory of the Specialist Hospital Yola, Nigeria and Laboratory isolates of *Cryptococcus neoformans* and *Candida albicans* obtained from the Microbiology Laboratory of the Department of Microbiology, Federal University of Technology Yola, Nigeria. All the isolates were subcultured unto fresh Potato dextrose agar slants and stored at room temperature.

Preparation of crude extracts: Four hundred grammes (400g) of the powdered plant material in each batch was exhaustively extracted by soxhlet extraction method using 1000ml of either Petroleum ether, (A), or Methanol, (B), or 70% v/v Methanol in H₂O, (C). The solvent used in each batch was recovered under pressure until dry extracts were obtained and then stored separately in amber colored bottles labeled as A, B and C. The percent yield was 35.8% (petroleum ether, 22.5% (methanol) and 41.7% for methanol water.

Determination of phytochemical constituents: The freshly prepared extract was subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins and phenols as earlier described.⁵

Assay of antifungal activity: The paper disc diffusion method⁶ was used with slight modification. 10 ml Potato Dextrose Agar (PDA) (Difco) was dispensed into Petri dishes and allowed to solidify. Spores were recovered by gently swabbing the surface of the culture plates with a sterile cotton swab and the swab dipped in 5 ml sterile saline containing 0.1% Tween 80 in a test tube to suspend the spores. The suspended spores were diluted serially and adjusted to initial inoculum of 4×10^4 spores/ml by counting using a counting chamber. A micropipette was used to introduce 0.1ml of the spore suspensions (adjusted to 4×10^4 conidia/ml) on to the agar plate, and spread with glass rod spreader under sterile condi-

Table-I: Phytochemical constituents of root extracts of *Deuterium microcarpum*

Extract	Saponins	Tannins	Alkaloids	Flavonoids	Balsams	Anthraquinones
Petroleum ether	-	-	+	+	+	-
Methanol	+	+	+	+	+	-

Key: - = absent; + = present

tions. Sterilized discs (6mm, Whatman No 1) were prepared by soaking in different concentrations of the extracts (100, 200, 500, 1000, and 2000µg/ml) for 6 h in bijou bottles. The discs were then removed and allowed to dry in a sterile Petri dish, then stored in screw capped bottles for further use. To assay for antifungal activity, five of the discs impregnated with different concentrations of the extract were placed on a fungal spore seeded plate with the help of sterile forceps. For standard antifungal agents, sterilised discs (6mm, Whatman No 1) were also prepared by soaking in 100µg/ml concentrations of nystatin and fuchsin for 6 h in bijou bottles. The discs were then removed and allowed to dry in a sterile Petri dish and were also placed on the fungal spore seeded plate with the help of sterile forceps as positive control, while discs soaked in sterilised distilled water only without extracts were used as negative control. Three replicates were produced for each fungus. Culture plates containing *C. albicans* were incubated at 37 °C for 24 h while other culture plates containing the rest of the fungi were incubated at room temperature (32-35 °C) for 48-72 h. Antifungal activity was determined by measurement of the zone of inhibition (mm) around the discs after the period of incubation.

Determination of minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC): The MIC was determined using the agar dilution method⁷ with slight modifications. Varying concentrations (1000, 700, 500.0, 400.0, 300.0, 200.0, 100.0, 50.0, 25.0 and 12.5µg/ml), of the extracts were prepared and incorporated separately into respective PDA plates. All the plates were incubated at 25 °C for 48 h and inhibition of growth was noted. The MICs were recorded after 48 hour. To determine the MFC, plates which did not show any growth after 48 h from the MIC determination were further incubated for 72 hour and after incubation, the concentration at which no visible growth was seen was noted as the MFC.

RESULTS

Qualitative analysis for phytochemical constituents from this study revealed the presence of saponins, tannins, alkaloids, flavonoids and balsams (Table-I). The study revealed that *Deterium microcarpum* possessed significant activity against some pathogenic fungi with the methanol extracts demonstrating the highest activities of 22 and 20mm diameter of zone of inhibition against *T. mentagrophytes* and *P. digitatum* respectively, followed by those

Table-II: Antimicrobial activities of extracts of *Deterium microcarpum*

Organisms	Zone of inhibition (mm)																
	100			200			500			1000			2000			Amp B (10 µg/ml)	Nyst (10 µg/ml)
	MW	PE	ME	MW	PE	ME	MW	PE	ME	MW	PE	ME	MW	PE	ME		
<i>A. niger</i>	-	-	-	4	4	8	6	8	10	8	10	14	8	12	16	8	10
<i>P. digitatum</i>	-	-	-	4	8	8	8	10	14	10	14	16	10	16	20	10	12
<i>F. oxysporum</i>	-	-	-	6	6	8	6	8	10	8	10	12	10	12	16	18	16
<i>Cr. neoformans</i>	-	-	-	6	8	10	6	10	12	8	10	12	8	12	16	12	14
<i>T. mentagrophytes</i>	-	-	4	6	12	14	8	16	18	10	16	18	10	20	22	-	6
<i>C. albicans</i>	-	-	-	-	6	6	8	10	12	8	10	12	8	12	14	-	-

Key: MW → methanol in water extract; ME → methanol extract; PE → petroleum ether extract;
 - → no measurable zone; Amp B → amphotericin B; Nyst → Nystatin

against *A. niger*, *Cr. neoformans* and *F. oxysporum* (16mm zone diameter of inhibition in each case) at 2000µg/ml (Table-II). The least activity (14mm zone diameter of inhibition) was demonstrated against *C. albicans* at 2000 µg/ml. Methanol water extracts demonstrated the least activity. For the standard antifungal agents the highest activity (18 mm zone diameter of inhibition) was demonstrated by amphotericin B compared to that of nystatin (16 mm zone diameter of inhibition) against *F. oxysporum*. The least activities of 8 and 6 mm zone diameter of inhibition were demonstrated by amphotericin B and nystatin against *A. niger* and *T. mentagrophytes* respectively. *C. albicans* was resistant to both the two antifungal agents. Table-III shows the results of determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) the extracts of *D. microcarpum* and the standard antifungal agents amphotericin B and nystatin. Results showed that, MIC and MFC values for the extracts ranged between 100-200 µg/ml with the least MIC and MFC values of 100µg/ml each demonstrated against *P. digitatum* and *T. mentagrophytes* respectively, while the highest values of 700µg/ml (MIC) and 1000µg/ml (MFC) against *C. albicans* and 400µg/ml (MIC) and 700µg/ml (MFC) against *A. niger*. For the standard antifungal agents, MIC and MBC values ranged between 12.5-700µg/ml for amphotericin B and 25-400µg/ml for nystatin with amphotericin demonstrating the least MIC value (12.5µg/ml) against *F. oxysporum* and *Cr. neoformans* compared to nystatin (25µg/ml) against *Cr. neoformans*.

DISCUSSION

The exhibition of antifungal activity has been attributed to the presence of plant bioactive compounds (Table-I) which are employed as natural defense mechanisms against pathogenic bacteria, fungi, viruses and pests.⁸ All the fungi including *A. niger* were reasonably susceptible to the extracts (Table). This outcome is contrary to earlier reports⁹⁻¹² in which the organism was resistant to all the extracts and antifungal agents tested.⁹⁻¹² Amphotericin B (AMB) is considered the "gold standard" for treatment of these infections. AMB is however associated with a number of severe and sometimes life threatening side effects including fever, chills and nephrotoxicity. Other treatment regimens include the 'azole' antifungal drugs, of which fluconazole and itraconazole are the most widely used. There is however lack of efficacy of and the emergence of resistance to itraconazole and also, limited efficacy of itraconazole against pulmonary aspergillosis. This highlights the need for new antifungal agents with potent and broad spectrum fungicidal activities for the effective management of these infections.¹³ The extracts were active against agents of invasive fungal infections which are important causes of death in severely immunocompromised patients and since the early 1990s invasive aspergillosis has become predominant.¹⁴ Resistance demonstrated by these fungi in previous investigations encouraged the use of drug combinations (amphotericin B (AMB) and rifampin (RIF)) to investigate antifungal activity.¹² The fungi used in this study are causative agents of various mycotic

Table-III: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the methanolic extracts of *Deterium microcarpum* against the test organisms.

Organisms	Extract	Standard antifungals				
		Amphotericin B		Nystatin		
		MIC (µg/ml)	MFC (µg/ml)	MIC (µg/ml)	MFC (µg/ml)	
<i>A. niger</i>	400	700	25	25	200	200
<i>P. digitatum</i>	100	300	100	100	200	200
<i>F. oxysporum</i>	400	500	12.5	25	50	50
<i>Cr. neoformans</i>	200	300	12.5	50	25	50
<i>T. mentagrophytes</i>	100	100	400	700	300	400
<i>C. albicans</i>	700	1000	500	500	50	50

infections; *C. albicans* (Candidiasis), *Cr. neoformans* (cryptococcosis), *A. niger* (systemic mycosis-aspergillosis) and *T. mentagrophytes* (ringworm). The higher MBC values to the corresponding MIC values from our data (Table-III) suggest that the fungal agents could be fungistatic at lower concentrations. Novel antifungal substances can be sourced from *Deterium microcarpum* for possible development of antifungal drugs for the treatment of systemic and superficial mycotic infections. Further studies on a wide range of bacteria and fungi should however be carried out.

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