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Antifungal Activity of the Methanol Tuber Extract of *Dioscorea Dumetorum (Pax)*

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Abstract
The antifungal activity of the methanol tuber extract of *Dioscorea dumetorum (Pax)* was investigated using standard techniques. The phytochemical screening revealed the presence of biologically active secondary metabolites (alkaloid, tannin, saponin and flavonoid). The methanol crude extract contained 19.4% of tannin, 2.8% of alkaloid, and 0.9% Saponin. These secondary metabolites have been reported by various authors to possess significant antimicrobial activities Okezie *et al*., 2016; Eleazu *et al*., 2013; Usman and Osuji, 2007; Prakash and Hosetti, 2010.The extract exhibited various antifungal activities in a dose dependent manner producing an inhibition of 8, 6, 4, & 2 mm against *Tinea spp.1* at concentration of 400, 200, 100, and 50 mg/mL respectively. Also, the extract exhibited activity against *Tinea spp.2* and *Candida albicans* having, a minimum inhibitory concentration of 100 and 200 mg/mL respectively. At a concentration of 400 mg/mL, the extract inhibited most of the test organism producing an inhibition zone of 8, 5, 4, 3, and 3 mm against *Tinea spp.1*, *Tinea spp.2*, *Candida albicans*, *Aspergillus niger* and *Tinea spp.4* respectively. No antifungal activity was recorded against *Tinea spp3*, *Tinea spp5* and *Tinea spp6*. Results of this study suggest that methanol extract of *Dioscorea dumetorum (Pax*) could be a promising source of novel antifungal compounds with pharmaceutical and industrial importance.

Keywords: Dermatophyte, Candida albicans, Aspergillus niger, Dioscorea dumetorum (Pax), Phytochemical screening, Secondary metabolites

Introduction
The incidence of fungal infections is observed to be more and more difficult to treat with existing drugs (Weitzman and Summerbell, 1995; Straten, 2003), this is as a result of fungal infections in humans becoming asymptomatic or less virulent especially in immunocompromised persons. Therefore, there is an urgent need for novel and useful antifungal agents to provide assistance and relief in both systemic and topical fungal infection. Human fungal pathogens are prone to develop ‘‘drug’’ resistances and in part increased the incidence of dermatophyte and *Candida albicans* infections worldwide. Studies have shown that immunocompromised persons have higher risk of such infections (Straten *et al*., 2003)**.** Presently, antifungal drugs used in treatment of such infections have several adverse effects, like toxicity, emergence of resistant strains, cost etc. Consequently, in recent years, research focuses on alternative source of antifungal drugs (Svetaz *et al*., 2010); that will address safety and environmental problems.
Medicinal plants represent a rich source of antimicrobial agents (Srivastava *et al.,* 1996). Yams, *Dioscoreacea* (genus; *Diocorea*) are staple tubers of West African origin IITA (1993). Botanically, they are flowering plants with about 600 species, found mainly in the tropical and warm temperate regions in the world. Some are cultivated for their edible tubers; while the wild species are valuable famine food and other species are sources of drugs both in traditional and western medicines (Eka, 1998). Some commercially and nutritionally important varieties of yam tubers are *D. rotundata* (white yam), *D. alata* (water yam), *D. bulbifera* (aerial yam), *D. opposita* (Chinese yam), *D. cayenensis* (yellow yam) and *D. dumentorum* (trifoliate yam).Yams play a significant role in the diet of most Africans, the Caribbean and South Pacific where they have been reported to represent about 12% of the

food consumed (FAO, 2008; Alozie *et al.,* 2009). *D. dumetorum (Pax)* is one of the six species of yam cultivated in Nigeria. It is an important food security crop and is mostly consumed in West Africa (Sefa-Dedeh and Afoakwa, 2002)). It originated in tropical Africa and occurs in both wild and cultivated forms. The local names in Nigeria are: *Kosanrogo* in Hausa, *Ona* in Ibo and *Esuru* in Yoruba. The wild varieties are commonly used for pharmaceutical or medicinal purposes by African and Asian people (McAnuff *et al*., 2014). Literature survey reveals the importance of *Dioscorea species* in fulfilling the dietary and medicinal requirements such as the use of its root extract for treating cholera, constipation, sores, piles and skin diseases. (Foster and Duke, 2000; Natraj *et. al.*, 2009). Also, extracts of *Dioscorea species* have been reported to contain significant amounts of bioactive secondary metabolites such as the (phenolics, tannins, flavonoid, and saponin) reported to have antifungal activity (Eleazu *et al.*, 2013; Jayshree and Dhruva, 2013). The present investigation was undertaken to evaluate the antifungal activity of methanol extract of *Dioscorea dumetorum* (*Pax*), collected from Ihiala farmland, Anambra-Nigeria.

Materials and Metods

Fig.1: Dioscorea dumetorum (Pax)

Plant material and extraction

Fresh tubers of *Dioscorea dumetorum* (Pax) were harvested in October, 2015 and identified on the basis of their morphological characteristics. The identity was confirmed by a botanist in the faculty of Pharmaceutical Sciences Nnamdi Azikiwe University, Awka-Nigeria. The powder of the tuber samples (432 g) was extracted with methanol (cold maceration) (Hayet *et al*., 2008). The extract was filtered and concentrated at 40°C using water bath Genlab Limited, UK**).** Test samples were prepared in dimethyl sulphoxide (DMSO) and graded dilutions were made.

Phytochemical Screening

The phytochemical analyses of the crude methanol extract was carried out in order to ascertain the presence of its constituents such as flavonoids, alkaloids, saponins, steroids, tannins, cardio-active glycosides, proteins, and carbohydrate using standard methods of analyses (Harborne, 1973; Sofowora, 1993; Trease and Evans, 2002).

Organisms

The isolates used in this study were obtained from the Department of Pharmaceutical Microbiology & Biotechnology, Nnamdi Azikiwe University, Awka-Nigeria.

Dermatophyte culture

The cultures of dermatophyte species Tinea spp 1, Tinea spp 2, Tinea spp 3, Tinea spp 4, Tinea spp 5, Tinea spp. 6
/

Candida albicans and Aspergillus niger cultures

Candida albicans and Aspergillus niger.

Antimicrobial assay

The antifungal activity was conducted using the method earlier described by (Jayshrhee & Dhruva, 2013), with little modification by Kaushik & Goyal (2008). A sterile swab was used to aseptically inoculate 150 µl of the inoculums (2.5 × 104 c.f.u. mL-1) of each of the fungal suspension (*Dermatophyte, C. albicans* and *A. niger*) on the surface of sterilized sabouraud dextrose agar. The tests were carried out using a stock concentration of 400 mg/ml prepared by dissolving 4 g of the crude extract into 10 ml of sterile distilled water. Thereafter, graded concentrations of (200, 100, 50, and 25 mg/mL) were made using DMSO as the diluent). A well of 8 mm diameter was made in the agar plate then loaded with 100 µl of each of the concentration of the test sample and incubated at 28±2°C for 15-20 days. The inhibition zone was observed and then recorded in millimeters using a transparent metre rule. The test was conducted in triplicate and results presented as mean ± SEM. Griseofulvin 50 µg and Miconazole 50 µg served as the Standard positive control against the *dermatophytes* and the (*C. albicans and A. niger*) respectively.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test plates. The MICs of the active extracts were determined by agar dilution method described earlier with some modifications. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, a stock solution of the crude extract (4,000 mg/mL) was made then 2-fold serial dilution was done to get graded concentrations (2,000, 1,000, 500, 250 mg/mL) of the extract. Then 1 mL of each of these concentration was transferred into a sterile petri dish and properly mixed with 9 ml of molten sabouraud dextrose agar previously cooled to 45-50^{O}C. After a proper mixing is done the final concentrations becomes 400, 200, 100, 50, and 25 mg/mL respectively. Finally, the different test organism is streaked on the solidified agar properly labeled and incubated at 28±2°C for 15-20 days. The MIC was interpreted as the lowest concentration of the test sample that inhibited visible mycelia growth. Each experiment was performed in triplicate.

Statistical analysis

Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 20.0. Results were presented as the means ± standard error mean of triplicate experiments. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P<0.05$.

Results and Discussions

The phytochemical screening of the crude methanol leaf extracts of *Dioscorea dumetorum Pax* (Figure 2) revealed the presence of flavonoids, tannins, terpenes, Saponin, protein, and carbohydrate; cardiac glycosides and steroid were found to be absent. These classes of secondary metabolites are known to show curative activity against several pathogens and therefore could explain its use traditionally for the treatment of wide array of illnesses (Hassan *et al.*, 2004; Usman, *et al*; 2005).

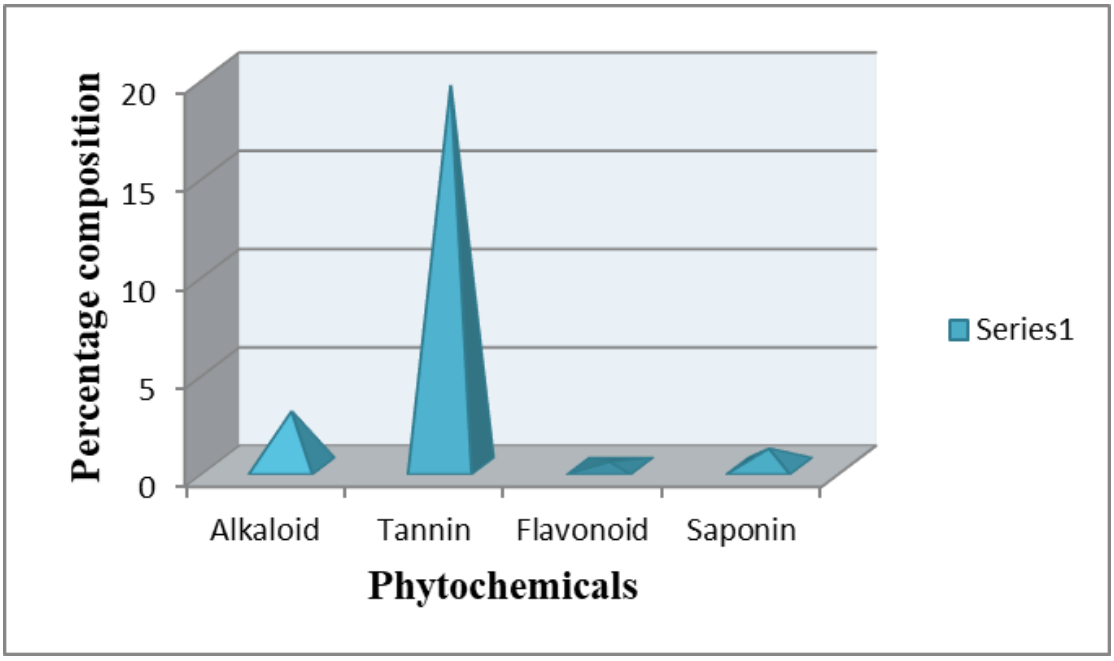

Fig. 2: Phytochemical composition of the tuber extract of *Dioscorea dumetorum Pax*

The *in vitro* antifungal screening presented in Table 1 showed the susceptibility of most of the *dermatophyte spp*. as well as the yeast and mould to the methanol crude extract of *Dioscorea dumetorum (Pax).* The result (Table 1) showed that the extract exhibited considerable level of inhibition against *T.ssp1, T.ssp2, A. niger* and *C. albican* isolates. At 400 mg/mL the extract demonstrated an interesting activity against *T.ssp1, T.ssp 2, A. niger* and *C. albican* with an IZD of 8, 5, 3, and 4 mm respectively. The extract demonstrated the best activity against *Tinea species1* with an MIC of 50 mg/mL. *T.ssp3, T.ssp5 and T.ssp6* were resistant to the extract at the tested concentrations.

The positive controls produced larger inhibition zones statistically significant ranging between 14 – 25 mm against the test organisms in relation to those activities produced by the extract against the organisms under study. However, it is suggested that diameters of zones of inhibition of this extract could not be unrelated to the presence of some important plant secondary metabolites such as (alkaloid, flavonoid, saponin, tannin and terpenoids) detected in the extract (Table 2) capable of acting against various cell components of both the *dermatophyte, Candida albicans* and *A. niger* thereby agreeing and authenticating the ethnomedicinal/folkloric claims of decoctions of this plant (tuber) against various ailments/infectious diseases (Foster and Duke, 2000; Natraj *et. al.*, 2009).

Table 1: Antifungal activity of the methanol crude extract of *Dioscorea dumetorum Pax* tuber.

Test Organisms	Concentration (mg/mL) / IZD (mm)					Positive control
	400	200	100	50	25	Griseofulvin 50 µg
Tinea spp 1	8±0	6±0	4±0	2±0	0±0	20
Tinea spp 2	5±0	3±0	2±0	0±0	0±0	18
Tinea spp 3	0±0	0±0	0±0	0±0	0±0	23
Tinea spp 4	2±0	0±0	0±0	0±0	0±0	14
Tinea spp 5	0±0	0±0	0±0	0±0	0±0	18
Tinea spp 6	0±0	0±0	0±0	0±0	0±0	15
						Miconazole 50 µg
A.niger	3±0	0±0	0±0	0±0	0±0	17
C.albicans	4±0	2±0	0±0	0±0	0±0	14

Result expressed as mean ± standard error mean.
Negative control used was DMSO; No activity was recorded.

Table 2: Result of the Minimum inhibitory concentration of the methanol crude extract of *Dioscorea dumetorum Pax* tuber

Test Organisms	Concentration (mg/mL)				
	400	200	100	50	25
Tinea spp 1	-	-	-	-	+
Tinea spp 2	-	-	-	+	+

Tinea spp 3	+	+	+	+	+
Tinea spp 4	-	+	+	+	+
Tinea spp 5	+	+	+	+	+
Tinea spp 6	+	+	+	+	+
A.niger	-	+	+	+	+
C.albicans	-	-	+	+	+

Key: + = growth; - = no growth

The result of the Phytochemical screening reveals that there were more secondary metabolites such as alkaloid (2.8%) and tannin (19.4%) present in the methanol crude extract of *D. dumetorum Pax* (Fig. 2) compared to that present in the extracts of *Dioscorea alata* (0.25%);(2.18%) and *D. bulbifera* (0.38%);(2.21%) respectively as reported by Eleazu *et al*., (2013). These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties (Molan & Faraj *et al*., 2010; Zongo *et al*., 2011). Thus, the antimicrobial properties of the methanol extract of *Dioscorea dumetorum Pax* may be attributed to the presence of these secondary metabolites. This is in line with (Okwu, 2004) who reported the healing potentials of tannin-rich plants. Furthermore, the presence of Tannins in the extract was significantly high compared to other secondary metabolites detected in the extract. Tannins have been reported to bind to proteins, carbohydrates, gelatins and alkaloids, and are classified as active antimicrobial compounds (Prakash, (2010)**.**

Therefore, the potential antifungal activity demonstrated by the methanol crude extract of *Dioscorea dumetorum (Pax)* tuber may as well be attributed to the effect of the significantly high amount of tannins detected in it.

Minimum inhibitory concentration

From the results of the minimum inhibitory concentration (MIC) presented in (Tables 2), the different concentrations of the methanol extract of the tuber of *Dioscorea dumetorum Pax* investigated in this study, exhibited various antifungal actions in a dose dependent manner against the different fungi *(Dermatophyte, Candida albicans* and *Aspergillus niger*) tested in this study, when compared with the standard fungicide (Table 1)**.** At an MIC of 50, 100, and 200 mg/ml the *T.ssp1, T.ssp2* and *C. albican* isolates were inhibited respectively. At 50 mg/mL, only *T.spp1* was inhibited. From the results it can be observed that the extract demonstrated a broad spectrum activity inhibiting both yeast and mould. This broad spectrum antifungal activity of this extracts could not be unrelated to the presence of the plant secondary metabolites such as (phenolics) detected. Consequently, in line with these findings, it has been reported that Phenolics possess antifungal potentials (Eleazu *et al*., 2013); (Okezie *et al*., 2016).

Conclusion

In conclusion, this study shows that the methanol tuber extract of *Dioscorea dumetorum Pax* produced significant antifungal inhibitory activities against almost all the tested organisms particularly the *dermatophyte species* (though not comparable) to the reference drugs.

Therefore, the study underscores the need for the purification and characterization of bioactive constituent(s) from the methanol extract of this tuber species (*Dioscorea dumetorum Pax*.) as possible antifungal agents.

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