EVALUATION OF THE ANTI-FUNGAL PROPERTIES OF EXTRACTS OF *DANIELLA OLIVERI*

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ABSTRACT

The *in vitro* anti-fungal activity of leaf and stem bark of *Daniella oliveri* Rolfe was investigated against selected yeasts and moulds including dermatophytes. Water and methanol were used to extract the powdered leaf and stem bark using cold infusion. Antimicrobial activity was assessed by agar-well diffusion. Phytochemical analysis was carried out using standard procedures. The plant extracts were active against the test organisms at concentrations ranging from 3.125-100 mg/mL. The methanol extracts were more active than the aqueous extracts with the highest inhibition against the yeasts, *Candida albicans* and *Candida krusei* (MIC values of 3.125 mg/mL and 6.25 mg/mL respectively). *Epidermophyton floccosum* and *Trichophyton interdigitale* were the least inhibited of all the fungal strains. Phytochemical screening revealed the presence of tannins, anthraquinones, flavonoids, cardiac glycosides, alkaloids and saponins. The anti-fungal activity of *Daniella oliveri* as shown in this study indicates that the plant has the potential of utilisation in the development of chemotherapeutic agents for the treatment of relevant fungal infections.

Keywords: *Daniella oliveri*, leaf and stem bark, anti-fungal activity, secondary metabolites

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INTRODUCTION

From time immemorial, medicinal plants have been found to be of important therapeutic aid for various ailments and diseases. Almost all cultures have depended partially or fully on herbal medicine because of its availability, efficacy, affordability, low toxicity and acceptability. The recurring problem of resistance of microorganisms to orthodox medicines has led to an increased interest in herbal products as sources of novel compounds to combat the emergence of newer diseases while preventing the resurgence of older ones (Akharaiyi and Boboye, 2010).

In Nigeria, many plants are used in ethnomedicine for the treatment of variety of diseases, one of which is *Daniella oliveri* Rolfe (Fabaceae), commonly known as West African copal tree, African copaiba balsam, Ilorin balsam, Accra copal and Benin gum copal. The plant is found in both temperate and tropical regions of the world, Amazon region, South America and Africa (Langenhein, 1973; Gentry, 1993). It is an indigenous plant of Africa found extensively in Benin, Cameroon, Gambia and Nigeria.

The leaves, stem bark and trunk of *D. oliveri* produce liquid oleoresin which consists of large but varying amounts of volatile oils, non-volatile resinous substances and small quantities of acids (Gilbert, 2000). The oleoresin is used in traditional medicine in the treatment of skin ailments, inflammation and genito-urinary tract diseases (Raffauf, 1992). It is also used as an antiseptic, antibacterial, laxative, purgative, diuretic and hypotensive agent (Fleury, 1997). The leaf, stem bark and root of *D. oliveri* are used to treat ringworm, scrotal elephantiasis, dysentery, syphilis, typhoid fever and ear ache (Nwaeze and Abariku, 2006). Decoction of leaf and bark is used as a mouthwash for toothache and tooth troubles. Young leafy shoots are pounded to a paste and applied on wounds to arrest bleeding and hasten healing. Leaf sap is taken by Tiv people in Nigeria as a cough medicine. The gum exudate from the bark is applied externally to treat itching skin and skin diseases (Burkill, 1997).

Even though there is an extensive information on the ethno-pharmacological uses of *D. oliveri*, there is a dearth of scientific reports on the anti-fungal properties of the plant, thus, the present study was aimed at evaluating the antimicrobial effect of the plant against selected fungal strains in order to ascertain its ethno-medicinal use in the treatment skin ailments and diseases.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves and stem bark of *D. oliveri* were collected in May 2013 within the evening hours in Ilemona village, Oyun local government, Kwara state, Nigeria. The taxonomical identification of the plant was done in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.

Preparation of plant materials

Freshly harvested leaves and stem bark of *D. oliveri* were thoroughly rinsed with clean water and air dried at 28 ^{0}C over a period of 7 days. The dried plant parts were pulverized with an electrical grinder.

Phytochemical screening

The powdered plant parts were screened for secondary metabolites using standard procedures (Sofowora, 1993).

Preparation of extract

The powdered leaves (500g) and stem bark (1000g) were soaked separately in 4000mL and 4500mL of methanol respectively using a 5L Erlenmeyer flask. The same procedure was carried out with water as extracting solvent. The flasks were corked and placed in a laboratory shaker for 4h and left for 7 days after which the extracts were decanted and filtered with Whatman No 1 filter paper. The filtrates were concentrated and dried on water-bath to obtain dry extracts.

Microorganisms

The fungal strains used for the study were pure cultures obtained from laboratory stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. They were, *Aspergillus niger, Candida albicans, Candida krusei, Rhizopus stolonifer, Epidermophyton floccosum, Trichophyton floccosum, Trichophyton interdigitale and Trichophyton rubrum*.

Anti-fungal bioassay

The anti-fungal activity of the extracts of *D. oliveri* was determined using agar-well diffusion technique in which 0.1 mL of 24 h to 5 days culture (equivalent to 1.5×10^6 cfu/mL) was used to seed Saboraud dextrose agar plates. A sterile cork borer (8mm diameter) was used to punch equidistant wells in the seeded agar medium. Each well was filled with graded concentrations of the reconstituted extract (3.125-100 mg/mL). The plates were incubated for 48 h to 5 days for moulds at 25 ^{0}C and 24-48 h for *Candida spp* at 35 ^{0}C. The diameter of zone of growth inhibition was measured and compared with the zones produced by the standard antibiotics (ketoconazole and tioconazole).

Minimum Inhibitory Concentration (MIC)

The broth dilution assay was used to determine the MIC of the plant extracts. The crude extracts were dissolved in di-methyl sulphur oxide (DMSO) and the solution obtained was added to sterile Mueller Hinton broth (MHB) to obtain a stock concentration of 400 mg/mL which was then serially diluted two-fold to obtain concentration ranges of 0.78 - 400 mg/mL. 5 mL of the plant extract solution was added aseptically to 5 mL of double strength MHB in the first test tube and mixed by shaking. 5 mL of the mixture was transferred to the second test tube which contained 5 mL single strength broth medium and the procedure was repeated till the last concentration was achieved. All the test tubes were inoculated with 0.1 mL inoculum (standardised at 1.5×10^6 cfu/mL). A test tube containing 5 mL MHB and 0.1 mL inoculum without plant extract served as a fertility control for the viability of the test organism. The test tubes were incubated for 48 h to 5 days for moulds at $25\ ^0$C and 24 - 48 h for *Candida spp* at $35\ ^0$C. The tubes were observed for turbidity and the lowest concentration that prevented fungal growth was taken as the MIC.

RESULTS AND DISCUSSION

The aqueous extraction of the leaf could not be completed as mould growth accompanied with foul odour was observed after 48 h of soaking in water. Phytochemicals detected in the plant were tannins, alkaloids, flavonoids, saponins, anthraquinones and glycosides. The leaf contained more bioactive constituents than the stem bark as tannins, anthraquinones and flavonoids present in the leaf were absent in the stem bark. Akharaiyi and Boboye (2010) reported the presence of saponins, tannins, anthraquinones, flavonoids and alkaloids in the plant parts of *D. oliveri*. Tannins, flavonoids, saponins and terpenes have shown medicinal and physiological activities (Edeoga *et al.,* 2005). Tannins, terpenes and terpenoids are reported to be toxic to bacteria, yeasts and filamentous fungi (Scalbert, 1991; Cowan, 1999).

The methanol leaf extract demonstrated remarkable antimicrobial activity against the moulds (*A. niger and R. stolonifer*) and the yeasts (*C. albicans* and *C. krusei)* with zone growth inhibition diameter (ZID) ranging from 10-26 mm, but had moderate activity against the other moulds (dermatophytes) with ZID range of 10-14 mm when compared with the standard drugs as shown in Table 1. There was no inhibition demonstrated by the leaf extract against *T. interdigitales* at a concentration of 100 mg/mL. Both standard drugs used for the study (ketoconazole and tioconazole) did not inhibit the growth of *T. interdigitales* and *E.floccosum*. It is significant to note that the fungal moulds which were sensitive to the plant extract were resistant to tioconazole.

Weak inhibitory activity was demonstrated by the aqueous stem bark extract, inhibiting only one microbial strain (*C. albicans*) at 100 mg/mL (Table 2). The methanol extract of stem bark however showed better activity than the aqueous extract with remarkable inhibitory activity against *C. albicans* and weak inhibitory activity against *R. stolonifer* and *E. floccosum* (Table 2). The MIC values for the leaf extract were lower for most of the test organisms than the stem bark extracts signifying a better activity with the leaf extract (Table 3). The lowest MIC value of 3.125 mg/mL was recorded for *C. albicans* and *T. rubrum*. Generally, extracts with MIC values below 8 mg/mL are considered to possess some antimicrobial activity (Fabry *et al*., 1998) and natural products with MIC values below 1 mg/mL are noteworthy (Rios and Recio, 2005).

C. albicans is a diploid fungus that causes opportunistic oral and genital infections in humans (Ryan and Ray, 2004), systemic fungal infections and candidemia which have caused morbidity and mortality in immune-compromised patients (Zeichner and Pappas, 2006). *T. rubrum* is the most common causative agent of dermatophytosis world-wide and has been reported as the main agent isolated in superficial mycoses in Brazil corresponding to almost 60% in all clinical cases (Esqunazi *et al.,* 2004; Cruz *et al*., 2007). The remarkable inhibitory effects of the plant extracts on the *Candida* species and the dermatophytes showed that the plant may contain constituents that may be useful in the management of the mycotic infections caused by these microorganisms.

Several bioactive compounds detected in the plant may have accounted for the anti-fungal activities observed while variations in the inhibitory potency of the plant parts may be due to variations in the concentrations of secondary metabolites in the plant parts.

CONCLUSION

Crude extracts of *D. oliveri* exhibited good antifungal activity which justifies the folkloric use of the plant in the treatment of certain skin ailments and some mycoses. The plant having

displayed promising biological activity can be useful in the formulation of topical applications for the treatment of relevant human and livestock fungal diseases.

Table 1: Anti-fungal activity of the methanol extract of the leaf of *D. oliveri*

Organism	Diameter of zones of growth inhibition (mm)							
	Concentration in mg/mL							
	100	50	25	12.5	6.25	3.125	Ketoconazole 10	Tioconazole 10
A.niger	13	12	11	-	-	-	19	-
C.albicans	27	26	24	18	14	12	20	20
C.krusei	13	11	11	10	10	-	12	10
R.stolonifer	13	14	11	11	10	-	13	-
E.floccosum	10	-	-	-	-	-	-	-
T.floccosum	12	12	12	11	10	-	18	19
T.interdigitale	-	-	-	-	-	-	-	-
T,rubrum	14	14	12	11	10	10	22	16

Key:

- : No inhibition

Table 2: Anti-fungal activity of the aqueous and methanol extracts of the stem bark of *D. oliveri*

Organism	Diameter of zones of growth inhibition (mm)											
	Concentration in mg/mL											
	Aqueous Extract				Methanol Extract						Ketoconazole	Tioconazole
	100	50	25	12.5	100	50	25	12.5	6.25	3.125	10	10
A.niger	-	-	-	-	20	16	14	10	10	-	20	-
C.albicans	19	-	-	-	23	20	23	18	15	13	20	20
C.krusei	-	-	-	-	19	14	12	-	-	-	12	10
R.stolonifer	-	-	-	-	11	-	-	-	-	-	13	-
E.floccosum	-	-	-	-	11	-	-	-	-	-	-	-
T.floccosum	-	-	-	-	15	13	11	-	-	-	18	19
T.interdigitale	-	-	-	-	14	9	-	-	-	-	-	-
T.rubrum	-	-	-	-	18	12	10	10	-	-	22	16

Key:

- : No inhibition

Table 3: Minimum Inhibitory Concentration (mg/mL) of the crude extracts of *D. oliveri*

Organism	Methanol leaf extract	Aqueous stem bark extract	Methanol stem bark extract
A.niger	12.5	200	6.25
C.albicans	3.125	25	3.125
C.krusei	6.25	100	12.5
R.stolonifer	6.25	200	50
E.floccosum	50	200	50
T.floccosum	6.25	200	12.5
T.interdigitale	200	200	50
T.rubrum	3.125	100	12.5

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