



Phytochemical Screening and Antifungal Activity of *Daniellia oliveri* Leaf Extracts Against *Trichophyton rubrum* and *Microsporum audouinii*

¹J.N. Keta., ²H.A Suberu., ³A.A. Aliero and ⁴K. Shehu

¹Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero,

²Department of Biological Sciences, Federal University, Lokoja

^{3&4}Department of Biological Sciences, Usmanu Danfodiyo, Sokoto

ABSTRACT

The *in-vitro* antifungal activity of *Daniellia oliveri* Rolfe leaf extracts was investigated against some selected dermatophytes (*Trichophyton rubrum* and *Microsporum audouinii*). Water and methanol were used to extract the powdered leaf using cold water method infusion at concentrations 25, 50 and 100% were tested against the fungal isolates. Antifungal activity was assessed by agar well diffusion method and phytochemical analysis was carried out using standard procedures. The plant extracts was found to be active against the test organisms at 25-100mg/ml as the ethanol extracts were found to be more active than the aqueous extracts with the highest inhibition against *T. rubrum* compared to *M. audouinii* with an MIC values of 3.125 and 200mg/ml respectively. *M. audouinii* was the least inhibited among the fungal strains. The antifungal activity of the extracts (water and ethanol) increased with increase in concentration. Analysis of Variance revealed higher significant inhibitory effects of both ethanol and aqueous extracts at both concentration 25, 50 and respectively 100% for the fungal isolates. There was significant difference in the grand mean inhibitory effect of both ethanol and aqueous extract on the test fungi at $P = 0.05$. The phytochemical screening revealed the presence of tannins, anthraquinones, flavonoids, cardiac glycosides, alkaloids and saponins. From the results obtained it can be concluded that the presence of the above phytochemical compound in *D. oliveri* may be responsible for the antifungal activities exhibited by the plants against the test organisms. The antifungal activity of *Daniellia oliveri* showed in this study indicated that the plant could be useful in the development of chemotherapeutic agents for the treatment of the potential fungal infections.

Keywords: *Daniellia oliveri*, Phytochemical, *Trichophyton rubrum* and *Microsporum audouinii*

INTRODUCTION

Traditional healers have long used plants to prevent or cure infectious diseases, Almost 50% of current pharmaceuticals are derived from the plant kingdom plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids and flavonoids which have been demonstrated to have *in vitro* antimicrobial properties (Gonzalez-Lamothe *et al.*, 2009). 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).

Daniellia 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 (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). The gum exudate from the bark is applied externally to treat itching skin and skin diseases (Burkill, 1997).

The dermatophytes cause lesion called dermatophytoses on the human being, these are the most frequent cutaneous mycosis. They appear in the skin (epiderm) and the dander (hair, fur nails). Dermatophytoses take various clinical aspects which makes a mycologic prelevement and profound diagnostic very important (Chabasse and Contet-Audonneau, 2011). The aimed of the study is to evaluate the antifungal activity of *Danielila oliveri* leaf extract against two dermatophytic fungi namely; *Trichophyton rubrum* and *Microsporum audouinii* and to analyses the phytochemical constituents of the plant leaf.

MATERIALS AND METHODS

Study Site

This study was conducted in Kebbi State and the study area is Jega Local Government Area, Kebbi State Nigeria. The

study area lies within latitude 10 – 13 °N and longitude 3.5 – 6 °E (Suleiman, 2008). The study area attains mean annual rainfall of 500mm to 1,300mm with a heavy concentration in the month of August. The wet season is followed by the dry season which commences from September to April.

Collection of Plant Materials

The fresh leaves branches of *Daniellia oliveri* were collected from bush farms at Rafin Guzuma in Jega L.G.A. The plant was authenticated by a plant Taxonomist Dr. Dharmendra Singh of the Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero and Voucher Number was issued as Department of Plant Science and Biotechnology Aliero (/KSUST /30).

Preparation and Extraction of Plant Materials

This was done according to the method as described by Galal *et al.* (2012). The leaves of *Daniellia oliveri* were shade-dried for seven days in a room. The dried plant parts were pounded with pestle and mortar, and a 25-mesh diameter sieve was used to obtain fine powder of each plant extract. The aqueous extract of the plant was prepared by placing 200g of the dried powder of the plant in one liter conical flask and 500ml of distilled water added. Then the mixture was stirred for 30 minutes by a magnetic stirrer (6000 rpm) and left for two days. The extract was then filtered through a fine cloth and again through filter paper (Whatman No, 1). The filtered materials was taken into a round bottom flask and then concentrated by evaporation of water from filtrates of the plant in water baths at 50°C (Rabiu *et al.*, 2015) till it reached the final volume of 10ml. After the evaporation of water from filtrates, the condensed extracts was preserved in tightly corked-labelled bottles and stored in a refrigerator until use.

Phytochemical Analysis

The plant extracts was tested for the presence of flavonoids, alkaloids, tannins, anthraquinones, saponins and cardiac glycosides using standard techniques described by Sofowora (1993).

Collection of Fungal Isolate

Five (5) primary schools were selected in Aliero metropolis, namely: University Staff Primary School, Attahiru model primary School, NYSC Nursery and Primary School, Muhammad Magawata model primary school and Aliero West Primary School respectively. A total of 25 infected Skin (5 each from the selected Schools) were collected from infected pupils with clinical manifestation of scalp ringworm (*Tinea capitis*). The sites of the infection were first cleaned with 70% v/v ethanol and light scraping. (Skin scales) samples were taken from the active age of lesion. All the samples were labeled appropriately in a coded brown envelopes and transported to Microbiology Laboratory, Kebbi State University of Science and Technology, Aliero for further analysis.

Ringworm Sample Processing

One gram (1g) of each sample was aseptically weighed and homogenized using sterile mortar and pestle for one minute, the serial dilution was conducted and 1g of each homogenized sample of scalp scrapings was placed in a sterile conical flask containing 10ml of sterile distilled water and mixed vigorously in order to obtain homogenized sample solution, 1-5 test tubes were filled with 9ml of sterile distilled water and placed in the test tube rack at vertical position (Cheesbrough, 2002).

Media Preparation

Sabouraud Dextrose Agar (SDA) was prepared according to manufacturer's specification. The medium was prepared by dissolving sixty-five gram (65g) of SDA powder into 1000ml of distilled water in conical flask and sealed with aluminium foiled paper and autoclaved at 121°C for 15 minutes, allowed to cool to 45°C before pouring 20 mls into each Petri dish and finally left over night for the confirmation of sterility before inoculation of the samples.

Culture Method

After 1 week of inoculation and incubation at room temperature (28-30°C), the plates was observed for colony growths and the colony types were noted and recorded (Campbell and Stewart, 2003). A fresh Sabouraud Dextrose Agar (SDA) were then prepared and used to subculture from the primary plates to obtain a pure culture. Using sterilized inoculating needles, a colony from the primary culture was cut and inoculated onto the fresh culture media plates. The inoculating needle was then sterilized by flaming to red hot after each cut to avoid the yield of mixed cultures. The plates were then incubated for another 1 week at room temperature. After 5-7 days incubation the plates were observed for pure colony growth (Campbell and Stewart, 2003).

Preparation of Aqueous Extracts

The aqueous extracts were prepared using Denis (2007) protocol, in which 15 g of each samples was soaked in 1L of distilled water, put to ebullition 20 mn, then the decoctions were kept overnight. The mothers' solutions were filtered and used to prepare the different treatments at the concentration of 10% (15 mg/ L).

Antifungal Susceptibility Test

The antifungal activity of *Daniellia oliveri* was determined by employing agar diffusion technique Sharma *et al.* (2011) for each isolate a suspension of mycelia from 9 days culture prepared in steril saline to a concentration of 10⁶ cells/ml the suspensions were swabbed evenly on the surface of the petri plates containing solidified sabouraud dextrose agar; 6mm diameter disks containing different concentration of the ethanolic and aqueous extract of *Daniellia oliveri* have been place on a gelose surface and let one hour in ambient temperature for diffusion, petris boxes have then been incubated at 27°C during 3 to 7 days, the inhibition zone diameters around the disks have been measured and recorded. The diameter of zone of growth

inhibition was measured and compared with the zones produced by the standard antibiotics (ketoconazole).

RESULTS

The results of the study conducted showed the morphological features of dermatophytes fungi isolated are presented in Table 1 and antifungal activity of aqueous Trace amount as shown in Table 3.

extracts of *Daniella oliveri* Rolfe tested at different concentration were showed in (Table 2). The results of phytochemical analyses of ethanol extracts indicated the presence of tannins, alkaloids, flavonoids, saponins, anthraquinones, glycosides, steroids, cardiac, saponin glycoside, volative oil and balsam. Saponin were obtained with highest

Table 1: Phytochemical Composition of the Ethanol Extract of *D. oliveri*

Phytochemical Constituents	Results
Tannins	++
Alkaloids	+
Flavonoids	++
Saponins	+++
Anthraquinones	+
Glycosides	++
Steroids	++
Cardiac	+
Saponin glycoside	++
Volative oil	++
Balsam	+

Key+ = small quantity, ++ = moderate quantity, +++ = large quantity

Table 2: Antifungal activity of the methanol of *D. oliveri* leaf extract

Conc.mg/ml	Diameter of zones of growth inhibition (mm)	
	<i>Trichophyton rubrum</i>	<i>Microsporium audouinii</i>
25	24.12	12.11
50	26.09	14.08
100	27.51	14.23
Ketoconazole	20.24	22.09
Water	-	-

Table 3: Antifungal Activities of Aqueous and Ethanol extract of *D. oliveri* leaf

Organisms	Zone of inhibition		
	Eth.extract (80mg/ml)	Aq.extract (80mg/ml)	Gs(160mg/ml)
<i>Trichophyton rubrum</i>	3.125	100.23	50.00
<i>Microsporium audouinii</i>	200.21	200.45	45.03

DISCUSSION

The crude extracts or purified form of plant has been used as medicines and cosmetics (Sofowora, 1993). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.*, 2007). In this study, the phytochemical screening of *D. oliveri* leaf extracts indicated the presence of tannins, alkaloids, saponins, cardiac glycosides, flavonoids, steroids, anthraquinones, saponin glycoside, volative oil and balsam. The presences of these secondary metabolites in *Daniellia oliveri* produce some biological activity in man and it is responsible for their uses as herbs medicinal. This is in agreement with the work done by Onwuliri *et al.* (2006). Also in agreement with the findings of Akharaiyi

and Boboye (2010) who reported the presence of saponins, tannins, anthraquinones, flavonoids and alkaloids in the plant parts of *D. oliveri*. According to Arun *et al.* (2010) the antimicrobial activities of the plant can be attributed to the presence of flavonoids and naphthoquinones. This statement disagree with the present study due to the absent of naphthoquinones. Flavonoid is used in medicine as antimicrobial, anti-inflammatory and anti oxidant (Evans, 1996). The antifungal potentials of *D. oliveri* plant extracts can be explained by the phytochemicals present (Emmanuel *et al.*, 2013). Ahmad *et al.* (1998) reported that phytochemical screening of these plants revealed the presence of alkaloids, saponins, tannins, flavonoids which have been found to form reversible complexes with fungal organisms. The variation in the inhibitory effects of the plant extracts could be due to the qualitative and

quantitative actions of the bioactive components present in the *D. oliveri*.

The low activities of the conventional antibiotics against the test organisms could be as a result of resistance of these organisms to the discs, possibly due to previous exposure to the antibiotics since the organisms were clinical strains. Fabry *et al.* (1998) also reported the antimicrobial resistance of all health care associated pathogen. This was also constancy by the fact that patients prefer to treat infections (particularly skin infections) using herbs rather than conventional methods (personal communication). Dermatophytes are a specialized group of fungi that causes a zoonotic skin infection of keratinized tissue, leading to skin eruptions which last for a longtime (Duraipandiyan and Ignacimuthu, 2011). The results of antifungal activities of *D. oliveria* aqueous and ethanol extracts showed promising anti-dermatophytic activity against *Trichophyton rubrum* and *Microsporum audouinii* from each concentration used in this study. This is in agreement with the findings of Hassan *et al.* (2007) in which aqueous extract of *P. tomentosa* was active against some fungal isolates including dermatophytes (*T. rubrum* and *T. mentagrophyte*). Although the value obtained in this study was higher, this could be attributed to the difference in extracting solvent and location of the plant.

CONCLUSION

The results obtained in this research revealed the presences of active phytochemical components that possesses antifungal activity against the selected demertophytes and could justifying its use in the treatment of skin and other related skin diseases.

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