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&4Department of Biological Sciences, Usman 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 (Gonzalaz-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).

*D. 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 dermatophyts 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 *Daniellia 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 olieveri 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 olieveri were shade-dried for seven days in a room. The dried plant parts were pounded with pistil and mortar, and a 25-mesh diameter sieved 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, tanins, 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 (Timea 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 envelops 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 obtained 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 were 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 olieveri was determined by employing agar diffusion technique Sharma et al. (2011) for each isolate a suspension of mycelia from 9 days culture prepared in sterile saline to a concentration of 106 cells/ml the suspensions were swabbed evenly on the surface of the petri plates containing solidified saboraud dextrose agar: 6mm diameter disks containing different concentration of the ethanolic and aqueous extract of Daniellia olieveri 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 extracts amount as shown in Table 3.

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

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac</td>
<td>+</td>
</tr>
<tr>
<td>Saponin glycoside</td>
<td>++</td>
</tr>
<tr>
<td>Volative oil</td>
<td>++</td>
</tr>
<tr>
<td>Balsam</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Conc.mg/ml</th>
<th>Trichophyton rubrum</th>
<th>Microsporum audouinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>24.12</td>
<td>12.11</td>
</tr>
<tr>
<td>50</td>
<td>26.09</td>
<td>14.08</td>
</tr>
<tr>
<td>100</td>
<td>27.51</td>
<td>14.23</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>20.24</td>
<td>22.09</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Eth.extract (80mg/ml)</th>
<th>Aq.extract (80mg/ml)</th>
<th>Gs(160mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton rubrum</td>
<td>3.125</td>
<td>100.23</td>
<td>50.00</td>
</tr>
<tr>
<td>Microsporum audouinii</td>
<td>200.21</td>
<td>200.45</td>
<td>45.03</td>
</tr>
</tbody>
</table>

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, volatile 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 threat 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. oliveri* 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 demerthophytes and could justifying its use in the treatment of skin and other related skin diseases.

**REFERENCES**


