



Comparative Study on Antifungal Effects of *Calotropis procera* and *Ficus gnaphalocarpa* Latex against *Tinea capitis*

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Abstract

This study was conducted to evaluate and compare the antifungal activities of *Calotropis procera* and *Ficus gnaphalocarpa* latex against three scalp ring worms; *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporum canis* using agar well diffusion method at 1.3 and 1.4ml latex concentrations. The plants latex was obtained at Rafin Guzuma and Giwa tazo village's farms at Maiyama Local Government Area of Kebbi State. Scalp ringworm samples were collected from pupils in (5) five Model Primary Schools within the study Area namely: Muhammadu Bello Dunbegu, Giwa tazo, Rafin Guzuma, Gidiga and Saran dosa Model Primary Schools. The antifungal assay results portrayed broad spectrum activity against the three dematophytes: *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporum canis* tested with comparable inhibitory zones ranged from 13.28 to 16.10mm for *C. procera* latex and 14.09 to 18.10mm for *F. gnaphalocarpa* latex as compared with the positive control (Griseofulvin). The study conducted revealed that, *F. gnaphalocarpa* latex has higher antifungal effect compared to *C. procera* latex on the test organisms.

Keywords: *Calotropis procera*, *Ficus gnaphalocarpa*, *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporum canis*.

INTRODUCTION

Traditional medicine has existed for ages and has relied largely on experience handed down from one generation to another (Ghani, 2003). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Chitme *et al.*, 2003; Mann *et al.*, 2008). Besides, the villages are far away from cities and mostly lack proper health facilities (Shinwari and Khan, 2000). Over sixty percent (60%) of Nigerian rural population depend on traditional medicine for their health care needs and the decoction made from some medicinal plants have been reported to be useful (Ajoku *et al.*, 2005). They provide virtually the only source of medicinally useful compounds for centuries. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide (Goyal and Mathur, 2011). The plant *Calotropis procera* (Tunfafiya) belongs to the family Asclepiadaceae. It is found distributed all over Africa, North Africa, and Southern Sahara, from Senegal to Central Africa Republic. All the parts, viz; root, stem, leaf and flowers of *C. procera* are in common used in indigenous system of medicine (Mukherjee *et al.*, 2000). The whole plant is toxic, with latex having strongest effect when used against cough and ringworm (Michel,

2004). In Northern Nigeria, particularly, Kebbi, Sokoto and Zamfara States, the latex, leaves, root, stem bark and fresh follicles of *C. procera* are used in indigenous practice to treat tropical fungal diseases, convulsion, asthma, cough and inflammation (Aliero *et al.*, 2001). The plant *Ficus gnaphalocarpa* belongs to the family Moraceae and found occurring in most tropical and Subtropical forests worldwide (Berg, 1999). The species have been documented to have anti oxidant, anti fungal, anti bacterial, anti-diabetes, antiviral, anti-protozoal, anticancer, cytotoxic, anti-ulcer, anti-inflammatory, anti-hyperglycemic, anti-diarrhea, hepato-protective, muco-protective and gastro-protective activity (Kuate *et al.*, 2011). Locally, the extract of *Ficus gnaphalocarpa* is used tropically for the treatment of *Tinea capitis* (ringworm) infections (Umar and Azare, 2006), *capitis* causes superficial infection of the skin of the scalp, eyebrows, and eyelashes, with a propensity for attacking hair shaft and follicles (Talaro, 2005). *Tinea capitis* is widespread in some urban areas, particularly in children, in North and South America, Central America, and Afro Caribbean (Umar and Azare, 2006). The infectious diseases caused by this fungus *Tinea capitis* is common in some countries especially Nigeria (Thakur, 2013).

T. capitis infection has been one among the most commonly diagnosed dermatophytosis of childhood and more frequently seen among prepubescent children (Mohrenschlager *et al.*, 2005). The two most common genera of dermatophytes responsible for *Tinea capitis* infection are *Trichophyton tonsurans* and *Microsporum canis*, with *T. tonsurans* as the most common cause of *Tinea capitis* in the United States (Elewski, 2008). However, *M. canis* is increasing in incidence in some parts of Europe and the United States (Binder *et al.*, 2009).

Several plants have been shown to contain some significant amount of antifungal activity on a wide range of microorganisms (Mossa *et al.*, 1991). In Northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery (Umar and Azare, 2006). The latex extracts of *Calotropis procera* and *Ficus gnaphalocarpa* which are well known to the people of Maiyama community in the study area are used locally for the treatment of *Tinea capitis* (ringworm) infections. Many drugs and medications have been shown to reduce swellings, redness and itching but not very effective (Mukherjee *et al.*, 2000). Antibiotics and antifungal gels or oral antibiotics are expensive and not readily available (Halder *et al.*, 2006). This research aimed at comparing the antifungal activities between *C. procera* and *F. gnaphalocarpa* latex extract against *Tinea capitis*.

MATERIAL AND METHODS

Study Area

The study was conducted at Maiyama Local Government Area of Kebbi State located on Latitude 12° 04' 56.10" N and Longitude: 4° 22' 8.65" E and falls within the Sudan Savanna region of the State with two major seasons: dry and rainy seasons and shares common borders with Suru and Bunza Local Governments from the West, Koko-Besse, from the South, and to the East is bordered by Jega in Kebbi State and Kebbe Local Government Area in Sokoto State. The topography is flat and slightly undulating with compact brown soil (NPC, 2006).

Ethical Issues

All schools used for this study were public schools with crowded classrooms and inadequate facilities. Majority of the pupils hailed from low income socio-economics class. Approval of this study was obtained from Maiyama Local Government Education Secretary, and the headmasters of the selected primary schools as well as Parent Teachers Association (P.T.A.).

Sample Collection

Five (5) primary schools were randomly selected from Maiyama Local Government area

namely; Muhammadu Bello Dunbegu Model Primary School Maiyama, Giwa Tazo Model Primary School, Rafin Guzuma Primary School, Gidiga Primary School and Saran Dosa Primary School. Ten (10) Pupils each within the age range 8-11 with clinical manifestation of scalp ringworm (*Tinea capitis*) were randomly sampled from each School. The sites of the infection were first cleaned with 70% v/v ethanol and light scraping was taken from the active lesion. All the samples were labelled appropriately in a coded brown envelopes and transported to Microbiology Laboratory, Kebbi State University of Science and Technology, Aliero for analysis.

Collection of Plant Materials

The fresh leaves branches containing flowers and fruits of *Calotropis procera* and *Ficus gnaphalocarpa* were collected from bush farms at Rafin Guzuma and Giwa tazo villages of Maiyama Local Government Area. The plant materials were authenticated by a plant Taxonomist in Department of Biological Sciences Kebbi State University of Science and Technology, Aliero and Voucher Numbers were issued, 03 and 511.

Extraction of *C. procera* and *Ficus gnaphalocarpa* latex

Fresh latex of *Calotropis procera* and *Ficus gnaphalocarpa* were collected from bush farms at Rafin Guzuma and Giwa tazo villages of Maiyama Local Government Area in June 2016. The latex was collected directly from the plants into a sterile specimen bottles by breaking the smooth stems of the plants and milky sap ooze out from the stems. This was repeated continuously until the required volume of (60ml each) latex was tapped as according to the method described by (Galal *et al.*, 2012; Rabiou *et al.*, 2015).

Serial Dilution of fungi

Five sterile test tubes were set in a test tube rack containing 9ml of distilled water each, 1ml of sample (scraps) was measured and transferred into the first test tube containing distilled water, using sterile pipette and sealed with cotton then shake vigorously, 1ml from first test tube were transferred into the second test tube and it was serially diluted to (10^{-5}) (Cheesbrough, 2006).

Culture Method

Test tube 3 and 5 of serial dilution were selected for inoculation, under aseptic condition using swab sticks. After seven days of inoculation and incubation at room temperature (28-30°C), the plates were observed for fungal growth and the isolates were identified and confirmed with clinical mycological atlas in accordance with Campbell *et al.* (1999).

Antifungal Analysis of plants latex extracts

The antifungal activity of the latex extracts was determined using agar well diffusion method. Sterile Sabouraud Dextrose Agar (SDA) was inoculated with fungal isolates by spread plate method. Wells were punched using cork borer (6mm) in the Ager and loaded with plant (latex) extracts and control wells containing Griseofulvin as positive control reference standard to determine the sensitivity of each tested fungal species and sterile distilled water was used as (negative control). The plates were allowed to stand for 15minutes on the bench to allow pre-diffusion of the latex extracts to take place and then incubated at 37°C for seven days and the antifungal activity was assessed by measuring the diameter of the zone of inhibition in millimeters by the use of transparent millimeter rule as described by Cheesbrough (2006). The SPSS Statistical Software was used for Statistical analysis. Average zone of inhibition was analyzed using one-way (ANOVA) with Tukey's multiple comparison testing to determine the significant differences between the control and experimental groups. All the comparisons were significant at P< 0.05.

RESULTS

Antifungal Activity of *Calotropis procera* Latex Extract

Table 1, Shows antifungal activity of *Caloreopsis procera* latex on the fungal isolates. It indicates that the positive control Griseofulvin (GSF) had the highest but not significant (p<0.05) zone of inhibition on *E. floccosum*, *T. rubrum* and *M. canis* with 19.0mm, 18.0mm and 19.0mm, respectively. Both the two concentrations of latex, 1.4ml and 1.3ml used on the test organisms, exhibited trend of antifungal activity. At 1.4ml concentration of the latex extract of *C. procera* 16.10mm was the highest zone of inhibition recorded on *T. rubrum* at significant (p<0.05) level, *E floccosum* had 15.14mm ranking second, while 14.11mm which was the least inhibition zone, observed on *M. canis*. Although, the inhibition zone values measured on *T. floccosum* and *M. canis* were found to be comparable, statistically.

At 1.3ml concentration of the latex, the highest and significant (p<0.05) zone of inhibition, 15.21mm was observed on *T. rubrum*, followed by *E. floccosum* 13.43mm and *M. Canis* which is the lowest with 13.28mm among the test organisms. The inhibition zones were not significantly different among *E. floccosum* and *M. canis*, but differed significantly with *T.rubrum*

Table 1: Antifungal Activity of *Calotropis procera* Latex Extract

Isolate	Concentration of latex (mg/ml)		Control +GSF
	1.3l	1.40	
<i>Epidermophyton floccosum</i>	13.43±0.5 ^b	15.14±0.1 ^b	19.0±0.0 ^a
<i>Trichophyton rubrum</i>	15.21±0.2 ^a	16.10±0.1 ^a	18.0±0.0 ^a
<i>Microsporium canis</i>	13.28±0.2 ^b	14.11±0.2 ^b	19.0±0.0 ^a

GSF = Griseofuvin (Positive control)

Table 2: Antifungal Activity of Latex Extract of *Ficus gnaphalocarpa*

Isolate	Concentration of latex (mg/ml)		Control +GSF
	1.30	1.40	
<i>Epidermophyton floccosum</i>	14.09±0.3 ^b	16.20±0.2 ^b	19.0±0.0 ^a
<i>Trichophyton rubrum</i>	16.10±0.2 ^a	17.10±0.1 ^b	18.0±0.0 ^a
<i>Microsporium canis</i>	17.12±0.1 ^a	18.10±0.1 ^a	19.02±0.0 ^a

GSF = Griseofuvin (Positive control), Negative control (Water) has no activity across. The results are presented in Mean and Standard deviation.

Table 3: Cultural and Microscopic Characteristic Fungal Isolates in all Primary School

Fungal	Cultural Characteristics	Microscopic Characteristic
<i>Microsporium canis</i>	White, coarsely fluffy, spreading, feathery texture.	Asymmetrical macroconidia, spherically shaped, cell walls thick and spherically roughened.
<i>Epidermophyton floccosum</i>	Greenish-brown, with a suede-like surface, raised and folded in the centre, with a flat periphery and submerged fringe of growth.	Smooth, thin-walled macroconidia produced in clusters growing directly from the hyphae.
<i>Trichophyton rubrum</i>	White colony.	Smooth-walled pyriform microconidia. Macroconidia borne laterally directly on hyphae and the hyphea are thick.

Antifungal Activity of Latex Extract of *Ficus gnaphalocarpa*

Table 2, shows antifungal activity of the latex of *Ficus gnaphalocarpa* against *E. floccosum*, *T. rubrum* and *M. canis*. The positive control drug (Griseofulvin) relatively showed higher diameter zones of inhibition than the latex of the two plants at 1.4ml and 1.3ml concentrations on tested fungal strains. The Positive control had 19.0mm, 18.00mm and 19.02mm inhibition zones for *E. floccosum*, *T. rubrum* and *M. canis* respectively. The values were statistically the same among the isolates. When 1.4ml of the latex was administered on the test isolates, result shows that 18.10 mm was the highest diameter zone of inhibition measured on *M. canis*, at significant ($p < 0.05$) level. It was closely followed by *T. rubrum* which had 17.10mm, the least and the lowest inhibition zone was recorded on *E. floccosum*, with 16.20mm. Inhibition zones were statistically same with the results in *E. floccosum* and *T. rubrum*.

At 1.3ml concentration of the latex, 17.12mm was the highest diameter zone of inhibition obtained on *M. canis*. *T. rubrum* had 16.10mm and ranked second while *E. floccosum* had 14.09mm which was the lowest and ranked third with the least zone of inhibition among the three isolates tested. The zones of inhibition observed on *E. floccosum* and *T. rubrum* were found to be the same statistically. It could be seen from the results that the highest concentration of the extract 1.4ml reported higher diameter zone of inhibition on the entire organisms tested in this study compared with 1.3ml, which was the lower concentration administered. *Ficus gnaphalocarpa* latex recorded higher zones of inhibition at various concentrations than *C. procera* latex.

Microscopic Identification of Fungal Isolates from Primary Schools

Table 3, shows the Colony and Microscopic identification of fungal isolates from Primary Schools covered in the study. All the isolates were identified on the basis of colony, morphology and cultural characteristics. The most occurring species identified include; *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Microsporium canis*. The three identified species were used to carry out antifungal sensitivity test of *C. procera* and *F. gnaphalocarpa* latex.

DISCUSSION

Various parts of *C. procera* species has been reported to be used in many countries for the treatment of varieties of diseases such as muscular spasm, joint pain, constipation, skin diseases (Mossa *et al.*, 1991). The results of the present study indicated that the latex of *C. procera* has antifungal potentials against ringworm. This finding agreed with that of Kuta (2008), who reported the same tradition of using *C. procera* extracts in Gwari communities of Niger State, Nigeria, for the treatment of ringworm which stimulated his interest in evaluating the aqueous extracts of the plant and found it to display a significant inhibitory effect on the dermatophytes tested even at low concentration.

The diameter zone of inhibition recorded in *C. procera* against the fungal isolates *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporium canis* ranged from 13.28mm to 16.10mm at significant level. While the diameter zone of inhibition with *F. gnaphalocarpa* latex extract ranged between 14.09mm and 18.10mm.

This is also in agreement with the findings of Kareem *et al.* (2008) who reported that the latex of *C. procera* possess significant inhibitory effect on fungal strains of *E. floccosum* and *M. canis* respectively.

The findings in this research are also in agreement with the research conducted by Halua and Vidyasagar (2012), who evaluated leaves extracts of two *Calotropis* species (*C. gigantean* and *C. procera*) using three different solvents against dermatophytes and *Aspergillus flavus* with chloroform extract having the highest inhibition observed. Similarly, *C. procera* was reported to have antifungal activity towards the three dermatophytes genera: *Miicrosporium* spp., *Trichophyton* spp. and *Epidermophyton* spp (Goyal *et al.*, 2013). Furthermore, Iqbal *et al.* (2015) reported the comparative efficacy of the chloroform and ethyl acetate extracts of *C. procera* leaf and latex which proved active against some dermatophytes and other pathogenic fungi. The latex of *F. gnaphalocarpa* recorded higher inhibition zone diameter (18.10 mm) against *M. canis* and lower inhibition zone (14.09mm) was reported against *Epidermophyton floccosum*.

The findings in this study showed that latex of *F. gnaphalocarpa* was more active in the treatment of scalp ringworm (*Tinea capitis*) than latex of *C. procera* as compared with control treatment (Griseofulvin). The therapeutic capability of the latex extracts may be due to pharmacological compounds such as flavonoids, anthraquinones, alkaloids or enzymes (Chevalier, 2001).

Higher antifungal activity of *C. procera* against *T. rubrum* as reported in this study agreed with

the findings of Saadabi *et al.* (2012), who also observed higher antifungal activity of *C. procera* aqueous extract against *Aspergillus fumigatus*, and *Altanaria solani*. Antifungal activity of *F. gnaphalocarpa* on fungal isolates as recorded in this study tallied with the report of Mbakwem-aniebo *et al.*, (2012), who investigated ethanolic leaf extract and latex of *Ficus carica* L. on *Candida albicans*, *Fusarium oxysporum* and *Aspergillus niger*. From the study conducted and the results obtained, on the effective comparative analysis of the antifungal effect of *Calotropis procera* and *Ficus gnaphalocarpa* latex extract against the test organism, it can be deduced that, both plants latexs showed effect against the test organism, but however, the study revealed that, *F. gnaphalocarpa* showed to have more antifungal effect on the test organism compared to *C. procera*. However, this clearly showed that, *F. gnaphalocarpa* has good antifungal property if effectively utilized.

Conclusion

The results and findings of the present study showed that *Calotropis procera* and *Ficus gnaphalocarpa* latex are effective anti-dermatophytic agents. The latex of both plants exhibited significant antifungal activity against *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporium canis* with comparable zones of inhibition. However, higher zone of inhibition was observed on the latex of *F. gnaphalocarpa* in all the tested fungal strains. It could be concluded that *F. gnaphalocarpa* latex is more effective than the latex of *C. procera*.

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