Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Article Info

Received: 14th May 2020

Revised: 7th June 2020

Accepted: 9th June 2020

[1]Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Kebbi State
[2]Department of Biological Sciences, Federal University, Birnin Kebbi
[3]Department of Biological Sciences, Federal University Lokoja, Kogi State
[4]Department of Microbiology, KSUST, Aliero

Corresponding author's email: Ketajb66@gmail.com

Cite this: *CaJoST*, 2020, 2, 148-152

Phytochemical and Antifungal Studies of *Calotropis procera* Latex against *Tinea capitis*

*[1]Keta, J. Naka, [2]Shehu Kasimu, [3]Suberu A. Hadi, [2]Aliero A. Aliyu, [1]Aminu Mubarak and [4]Keta M. Naka

This study was conducted to evaluate the antifungal activity of *Calotropis procera* latex against three scalp ring worms; *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporum canis* using agar well diffusion method at 1.31 and 1.40 ml latex concentrations. The plants latex was obtained at Rafin Guzuma farm in Maiyama Local Government Area of Kebbi State, Nigeria. Scalp ringworm samples were collected from pupils in (5) five Model Primary Schools within the study area namely; Muhammadu Bello Dunbegu Model Primary School, Giwa tazo Model Primary School, Rafin Guzuma Primary School, Gidiga Primary School and Saran Dosa Primary. The antifungal assay results portrayed broad spectrum activity against the three dematophytes: *Epidermophyton floccosum, Trichophyton rubrum* and *Microsporum canis* tested. The highest zone of inhibition was obtained from *Trichophyton rubrum* (16.10 ± 0.1^{a}) at concentration 1.40ml while the lowest was recorded in *Microsporum canis* (13.28 ± 0.2^{b}) at concentration 1.31ml. The phytochemical analyses conducted revealed the presence of alkaloid, glycoside, saponin, steroids, flavonoid, anthraquinone and volatile oils while cardiac glycosides, tannins, saponin glycosides and balsams were not detected. The presences of these phytochemical were inferred as being responsible for the antifungal activity of this plant. The results obtained indicate that *C. procera* latex is effective on the test organisms which further suggests the use of latex in the treatment of dermatophytosis especially *Tinea capitis* in which the plant latex was found to have greater effect.

Keywords: *Calotropis procera, Epidermophyton floccosum, Trichophyton rubrum, Microsporum canis and* phytochemical

1. Introduction

Calotropis procera commonly known as *Tunfafiya* in hausa speaking language belongs to the family Asclepiadaceous distributed all over Africa, North Africa and Southern Sahara, from Senegal to Central Africa Republic. *C. procera* (Ait.) is plant which is a soft-wooded, evergreen, perennial shrub with milky latex throughout. It has one or a few stems, few branches and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, furrowed, and light gray. A copious white sap flows whenever stems or leaves are cut. Giant milkweed has a very deep, stout taproot with few or no near-surface lateral roots. The opposite leaves are oblong obovate to nearly orbicular, short pointed to blunt at the apex and have very short petioles below a nearly clasping, heart-shaped base. The flowers are shallowly campanulate with five sepals that are 4 to 5 mm long, fleshy and variable in color from white to pink, often spotted or tinged with purple. The fruits are inflated, obliquely ovoid follicles that split and invert when mature to release flat, brown seeds with a tuft of white hairs at one end (Liogier, 1995). All the plant parts, viz; root, stem, leaf and flowers are in common used in indigenous system of medicine (Mukherjee *et al*., 2000). The whole plant is found to be toxic with latex having strongest effect when used against cough and ringworm (Michel, 2004). In Northern Nigeria, the latex, leaves, root, stem bark and fresh follicles of *C. procera* are used in indigenous practice to treat tropical fungal or skin diseases, convulsion, asthma, cough and inflammation (Aliero *et al.*, 2001).

The previous pharmacological studies include reports of anticancer, antifungal and insecticidal activity of *C. procera* (Ahmed *et al*., 2006). The flowers of the plant exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic, and antimicrobial effects and larvicidal activity. The latex of the plant is reported to possess analgesic and wound healing activity as well as anti-inflammatory and antimicrobial activity while the roots are reported to have antifertility and anti-ulcer effects (Yoganarasimhan, 2011).

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Plate 1. *Calotropis procera*

Tinea capitis is widespread in some urban areas, particularly among children, in North and South America, Central America and Afro Carabbean (Umar and Azare, 2006). The infectious diseases caused by this fungal organism *Tinea capitis* are common in some countries especially Nigeria and India (Thakur, 2013). *Capitis* causes superficial infection of the skin, scalp, eyebrows and eyelashes, with a propensity for attacking hair shaft and follicles (Talaro, 2005). The diseases or infections caused by this dematophyte has been among the most commonly diagnosed dermatophytosis of childhood infectious diseases and more frequently seen among prepubescent children (Mohrenschlager *et al.*, 2005). The two most common genera of dermatophytes responsible for *Tinea capitis* infection are *Trichophytonton surans* and *Microsporum canis* with *T. tonsurans* as the most common cause of *Ttinea capitis*.

Several plants have been shown to contain some significant amount of antifungal activity on a wide range of microorganisms (Mossa *et al,* 1991).The latex extracts of *Calotropis procera* which are well known to the people of Maiyama community in the study area and are used locally for the treatment of *Tinea capitis* (ringworm) infections. Many drugs and medications have been shown to reduce swellings, redness and itchiness but not very effective. Antibiotics and antifungal gels or oral antibiotics are expensive and not readily available (Halder *et al*., 2006). This research aimed at analysis the phytochemical properties and antifungal activities of *C. procera* latex extract against some selected *Tinea capitis.*

2. Materials and Methods

2.1 Study site

The study was conducted at Maiyama Local Government Area of Kebbi State. Maiyama L.G.A. situated at 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 respectively. Maiyama L.G.A has a minimum and maximum temperature of 23 to 42^{o}C .The topography is flat and slightly undulating with compact brown soil (NPC, 2006).

2.2 Ethical issues

All schools used for this study were public schools with crowded classrooms, populations 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.).

2.3 Collection of Test Organism (*Tinea capitis*)

Five (5) primary schools were randomly selected from Maiyama L.G.A 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 with average age 8 to11 years and having clinical manifestation of scalp ringworm (*Tinea capitis*) were 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 white plastic sample bottle and were brought to Botany Laboratory, Kebbi State University of Science and Technology; Aliero and kept in a refrigerator until further analysis.

2.4 Collection of Plant Material

The fresh leaves branches containing flowers and fruits of *Calotropis procera* were collected from bush farms at Rafin Guzuma village within Maiyama L.G.A. The plant was authenticated by a plant Taxonomist in the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero and Voucher Number was issued (KSUSTA/PSB/03).

2.5 Extraction of *Calotropis procera* latex

Water, ethanol 60% and chloroform were used during the extraction. The latex was collected directly from the plants into a sterile specimen bottles by breaking the smooth stems of the plants and collecting the milky sap in to the sample bottles. This was done until the required volume of 60 ml was obtained. This was repeated continuously until the required volume of 60ml (Rabiu *et al.,* 2015). The extracts were filtered and stored at 4^{o}C

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2.6 Photochemical Analysis

The *Calotropis procera* latex were analyzed for the presence of flavonoids, tannin, balsams, saponin, alkaloid, steroids, cardiac glycosides, anthraquinones, volatile oil and glycoside compounds using the standard colorimetric procedures as described by Sofowora (1993).

2.7 Antifungal Analysis of Plant 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. Wells were punched using cork borer (6 mm) in the agar 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 15 minutes on the bench to allow pre-diffusion of the latex extracts to take place and then incubated at 37^{o}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).

2.8 Statistical Analysis of Data

The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results were expressed as mean ± standard deviation of three replicates with Tukey's multiple comparison testing to determine the significant differences at P< 0.05 between the control and experimental groups.

3. Results and Discussion

3.1 Results

3.1.1 Phytochemical Screening

The result of phytochemical screening of *Calotropis procera* latex extract is presented in Table 1. Alkaloids, steroids, anthraquinones, glycosides, saponins, flavonoids and volatile oils were found to be present, while cardiac glycosides, tannins, saponin glycosides and balsams were not detected.

Table 1. Phytochemical composition of the *Calotropis procera* latex extract

Phytochemical	Inference
Flavonoids	++
Tannin	ND
Saponin	++
Alkanoids	+++
Glycoside	++
Steroids	+++
Cardiac glycoside	ND
Anthraquines	+++
Saponin glycoside	ND
Volatile oil	+
Balsam	ND

Key: (+++) = High concentration; (++) = Moderate concentration; + = Low concentration; ND = Not detected

3.1.2 Antifungal Activity

Antifungal activity of *Caloreopis procera latex* on the fungal isolates were presented in Table 2. 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 19.0 mm, 18.0 mm and 19.0 mm respectively. Both the two concentrations of latex, 1.4 ml and 1.3 ml used on the test organisms, exhibited trend of antifungal activity. At 1.4 ml concentration of the latex extract of *C. procera* 16.10 mm was the highest zone of inhibition recorded on *T. rubrum* at significant ($p<0.05$) level, *E flocosum* had 15.14 mm ranking second, while 14.11 mm 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 1.31 ml concentration, the highest and significant ($p<0.05$) zone of inhibition, 15.21 mm was observed on *T. rubrum*, followed by *E. floccosum* 13.43 mm and *M. Canis* which is the lowest with 13.28 mm among the test organisms. The inhibition zones were not significantly different among *E. floccosum* and *M. canis* but differed significantly with *T. rubrum.*

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Table 2: Antifungal Activity of *Calotropis procera* latex extract

Diameter of zone of Inhibition (mm)			
	Concentration (ml)		Control
Isolate	1.31	1.40	+GSF
Epidermophyton floccosum	13.43±0.5[b]	15.14±0.1[b]	19.0±0.0[a]
Trichophyton rubrum	15.21±0.2[a]	16.10±0.1[a]	18.0±0.0[a]
Microsporum canis	13.28±0.2[b]	14.11±0.2[b]	19.0±0.0[a]

The values are mean ± standard deviation. Values with different superscripts across the row are significantly ($p < 0.05$) different compared with the control; GSF = Griseofuvin (Positive control)

3.2 Discussion

3.2.1 Phytochemical

The result of the phytochemical screening of *Calotropi sprocera* latex in this study revealed the presence of alkaloids, glycosides, saponins, steroids, flavonoids, anthraquinone and volatile oils. The result obtained is in agreement with other reports analyzed on the leaves latex, stem and roots of *C. procera* by Hassan *et al*. (2006) and (Kuta, 2008). The results also in agreement with the finding of Kuta (2008) who reported the same traditional uses of the *C. procera* leaves 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. Additionally, Keta *et al*. (2018) reported the effect of *C. procera* latex on 3 dermatophytes (*Epidermophyton floccosum, Trichophyton rubrum* and *Microsporum canis*) at 1.31 ml and 1.40 ml concentrations.

3.2.2 Antifungal Activity

It has been reported that, various parts of *C. procera* has been used in many countries for the treatment of various diseases such as muscular spasm, joint pain, constipation and skin diseases (Mossa *et al*., 1991). However, in the present study, the latex of the *C. procera* was used to test the fungal activity of the plant. The results obtained from the data indicated that the latex of *C. procera* has antifungal potentials against the tests organisms.

The diameter zone of inhibition recorded in *C. procera* against the fungal isolates *Epidermophyton floccosum, Trichophytom rubrum* and *Microsporum canis* ranged from 13.28 mm to 16.10 mm which showed significant levels. This is in agreement with Kareem *et al*. (2008) who reported in his findings that the latex of *C. procera* possess significant inhibitory effect on fungal strains. The results obtained with that of Halua and Vidyasagar (2012) who similarly evaluated the effects of the leaves extracts of two *Calotropis* species *C. gigantean* and *C. procera* using three different solvent against dermatophytes and *Aspergillus flavus* in which chloroform extract was reported to have antifungal activity against the three dermatophytes genera: *Microsporum Trichophyton* and *Epidermophyton* species. Similarly, Iqbal *et al*. (2015), 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 higher antifungal activity of *C. procera* against *T. rubrum* as reported in this present research was also supported by Saadabi *et al*. (2012), who observed higher antifungal activity of *C. procera* aqueous extract against *Aspergillus fumigatus* and *Altanari asolani.* The result obtained suggests that *C. procera* has an active effect on the dematophyteic fungi especially *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum canis*.

4. Conclusion

The results of the present study show that *Calotropis procera* latex are effective antidermatophytic agents. The latex of this plants exhibited significant antifungal activity against *Epidermophyton floccosum*, *Trichophyton rubrum* and *Microscporum canis* with comparable zones of inhibition. Thus, the presences of these phytochemicals compound that possesses antifungal activity against the selected dermatophytes justified that latex of *C. procera* could be use in the treatment of *Tinea capitis*.

Acknowledgement

We are thankful to staff of Maiyama Local Government Education Authority, pupils (students) and laboratory Technicians of Kebbi State University of Science and Technology, Aliero for their kind support and encouragement.

Conflict of interest

The authors declare no conflict of interest.

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