Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20151012012909

Original Research

Efficacy and phytochemical analysis of latex of *Calotropis procera* against selected dermatophytes

Rabiu Muhammad Aliyu[1], Mikaeel Bala Abubakar[1], Adamu Bello Kasarawa[2], Yakubu Umar Dabai[1], Nafiu Lawal[1], Muhammad Bashir Bello[1], Aminu Yusuf Fardami[3]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria, [2]Department of Natural Sciences, Sokoto State Polytechnic, Sokoto, Nigeria, [3]Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria

Address for correspondence:
Rabiu Muhammad Aliyu, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.
E-mail: rabiuabdul2005@yahoo.com

Received: September 01, 2015
Accepted: October 04, 2015
Published: October 29, 2015

ABSTRACT

Background: Since ancient time, increased interest has been witnessed in the use of an alternative herbal medicine for managing, and the treatment of fungal diseases worldwide. This may be connected to the cost and relative toxicities of the available antifungal drugs. It has been a known tradition practiced in the northern part of Nigeria that parents and teachers use the white latex of *Calotropis procera* to treat *Tinea capitis* in children attending the local religious school in the area. This study was conducted in 2009 to ascertain the above claim. **Materials and Methods:** Fresh latex of *C. procera* was screened for their antifungal activity against species of dermatophytes: *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. using the agar incorporation method. **Results:** The result shows that the latex inhibits the *in vitro* growth of these pathogenic fungi to varying extents with *Trichophyton* spp. being the most susceptible ($P < 0.05$) and thus highly inhibited by the latex followed by the *Microsporum* spp. and *Epidermopyton* spp. was least inhibited. These inhibitions followed a dose-dependent trend as undiluted latex (100%) gave the highest inhibitory impacts ($P < 0.05$) when compared to serially diluted latex. The phytochemical analysis of the fresh latex indicated the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, and triterpenoids. **Conclusion:** The findings of this study confirmed the perceived usefulness of the latex in the treatment of *T. capitis* (ringworm) practiced in our society and therefore, its use topically in the treatment of dermatomycotic infection is encouraged.

KEY WORDS: Agar incorporation method, antifungal activity, dermatomycotic, latex

INTRODUCTION

Dermatophytes are a group of three fungal genera that can invade keratinized tissues and therefore able to cause superficial infections of the skin, nails and hair, thereby producing a disease referred to as ringworm in human and animals [1]. The infection is mostly cutaneous, i.e. restricted to the non-living cornified layer of the skin due to the innate inability of these groups of fungi to penetrate beyond the keratinized tissues or organs of the immunocompetent host [2].

The genus Calotropis (*Calotropis gigantea* and *Calotropis procera*) belongs to the family of Asclepiadaceae. These shrubs have been reported to exhibit a lot of medicinal properties which includes the antimicrobial, antimycotic, and anti-inflammatory effect [3].

C. procera is commonly called calotrope; other names are King's crown, kapok tree, Tumfafiya (in Hausa language), Bomubomu (Yoruba language). It is a spreading shrub with large grey-green leaves and large green inflated fruit similar in shape to a mango. A whitish sap (the latex) oozes out when the plant's stem is broken. The plant is native to tropical Africa and Asia. Studies from phytochemical analysis of *C. procera* suggest the presence of biologically active compounds such as Alkaloids, steroids, triterpenes; others include madaralbun, madarfluavil, caoutchouc, and calotropin [4].

The use of plant extracts for medicinal purposes is very widespread in the world, Nigeria inclusive. Many of these medicinal plants were being used against infectious disease causing agents, which are frequent nowadays, due to the emergence and increase in antimicrobial resistance and poor hygienic condition of our

environments. The increasing incidences of fungal infections coupled with the gradual rise in azole resistance and available antibiotics had highlighted the need to find more alternative antifungal agents from other sources [5]. Several plants have been shown to contain some significant amount of antifungal activity on a wide range of microorganisms [6]. The aqueous extract of the aerial part of *C. procera* is a prominent decoction used in Saudi Arabia for the treatment of varieties of diseases such as muscular spasm, joint pain, constipation, and fever [6]. Locally, the extract of *C. procera* is used topically for the treatment of ringworm [7]. In northern Nigeria, the latex, leaves, root, stem bark, and fresh follicles of *C. procera* were used in indigenous practice to treat topical fungal diseases, convulsion, asthma, cough and inflammation [8,9].

In Sokoto town (the area of the study), a study by Ameh and Okolo in 2004 [10] revealed the incidence of dermatomycosis among primary school pupils, and the study has attributed the observed incidence to the domestic animals as an important predisposing factor. It is evident from a survey (personal communication with the elderly people within the locality) that fresh latex of *C. procera* has been used from ancient time as a topical antimycotic treatment in the area.

This study was carried out to elucidate the antimycotic activity of the fresh latex of *C. procera* against selected representative species of the dermatophytes (the etiologic agent of dermatomycosis), and clarify the above claim by the local people.

MATERIALS AND METHODS

Plant Material

Fresh latex analysis of variance (the white liquid secretion) of *C. procera* was collected from the farmlands around Usmanu Danfodiyo University permanent site, Sokoto, Nigeria, in October 2009. The latex was collected into a sterile wide-necked screw-capped container by deliberately breaking the smooth stem and milky sap ooze out from the stem; this was repeated continuously until the required volume of the latex was tapped. The fresh latex was serially diluted 2-fold and 5-fold with sterile distilled water to give 50% and 20% of the original latex concentration respectively.

Phytochemical Analysis

The fresh latex of *C. procera* was sent to the Biochemistry laboratory of the department of Biochemistry, Faculty of Science, Usmanu Danfodiyo university, Sokoto for phytochemical analysis. The latex was analyzed for the presence of alkaloids, flavonoids, tannin, saponin, triterpenoids, anthraquinones, and glycosides compounds using the standard colorimetric procedures as described by Sofowora and Kennedy and Thorley [11,12].

The Fungal Species Used

Representative isolate each of *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. were used in this study. The isolates were kindly provided by Mr. Abdulrahman Barau of the Mycology laboratory of the Biological science department of the Usmanu Danfodiyo University, Sokoto.

Antifungal Susceptibility Testing

Fresh latex of *C. procera* was examined for its antifungal properties against *Trichophyton* spp., *Microsporum* spp. *and Epidermophyton* spp. - the causative agents of dermatomycosis (ringworm). Four dilution groups were prepared for this study, this includes a negative control (sterile distilled water instead of latex), original, fresh latex (100%), 2-fold serially diluted latex (50%) and 5-fold serially diluted latex (20%) groups.

The antifungal assay of the latex was conducted using the agar incorporation method as described by Taudou and Dwivedi and Dubey [13,14]. Briefly, the aforementioned concentrations (sterile distilled water, 100%, 50% and 20% latex) were aseptically mixed in a ratio of 1:3 with sterile sabouraud dextrose agar (SDA) and poured in 150 mm × 30 mm petri dishes, allowed to solidify and seeded in duplicates with fungal isolates previously cultivated on SDA. The inocula was aseptically cut with a sterile 10 mm cork borer, seeded in the middle of the petri dishes and incubated at 28°C-30°C in the dark. The growth of the dermatophytes on each culture plate was measured linearly (growth diameter) by the use of transparent millimeter rule daily for 6 days.

Statistical Analysis

An SPSS 20® statistical software was used for statistical analysis. The data generated in the study were presented in the form of tables using frequency distribution. Average daily mycelial growth was analyzed using one-way (ANOVA) with Tukey's multiple comparisons testing to determine the significant differences between the control and experimental groups. All comparisons were considered to be significant at $P < 0.05$.

RESULTS

The latex of *C. procera* was found to inhibit the *in vitro* growth of the three dermatophytic fungi studied to varying extents. Tables 1-3 show a measure of a diameter of mycelial spread for each of the fungi tested. Statistical analysis of the result shows that *Trichophyton* spp. was the most susceptible, and thus highly inhibited by the latex followed by the *Microsporum* spp. and *Epidermophyton* spp. was the least inhibited. It is observed that

Table 1: Measure of mycelial spread of *Trichophyton* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	5.6	32.6	55.6	81.0	86.6	89.6
Undiluted (100%)	6.7	7.5	14.0	21.4	21.1	39.7
2-fold dilution (50%)	6.8	7.4	15.2	20.2	30.7	45.8
5-fold dilution (20%)	5.6	10.2	12.3	25.7	35.7	51.6

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

100% (undiluted latex) of *C. procera* gave the highest inhibitory impact on the dermatophytes, whereas 20% latex recorded the lowest.

The diametric mycelial spread of the *Trichophyton* spp. ranges from 6.7 mm to 39.7 mm; the *Microsporum* spp. 7.3 mm to 65.1 mm and *Epidermophyton* spp., 8.3 mm to 72.7 mm. Statistical analysis shows that *Trichophyton* spp. was most susceptible, then *Microsporum* spp. and *Epidermophyton* spp. the least inhibited by the 100% latex ($P < 0.05$) [Table 1].

Similar trends of growth of the dermatophytes were recorded at the other concentration of 50, and 20% latex. However, the inhibition of their growth by the latex reduced with decreasing concentrations. All the three concentrations of the latex tested (i.e. 100, 50 and 20%) were significantly ($P < 0.05$) better than the control (standard). The result of this study indicated that *Trichophyton* spp. was the most sensitive, followed by *Microsporum* spp. while the *Epidrmophyton* spp. was the most resistant ones.

The result of the phytochemical analysis of the fresh latex of *C. procera* shows the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone and triterpenoids [Table 4].

Table 2: Measure of mycelial spread of *Microsporum* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	10.0	16.3	32.6	45.5	69.0	84.0
Undiluted (100%)	7.3	10.8	18.2	29.2	49.5	65.1
2-fold dilution (50%)	7.0	10.2	14.0	39.0	58.6	70.8
5-fold dilution (20%)	8.8	13.3	22.0	37.3	52.4	62.0

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

Table 3: Measure of mycelial spread of *Epidermophyton* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	15.0	30.0	46.5	72.0	84.0	88.5
Undiluted (100%)	8.3	20.7	38.3	52.7	61.2	72.7
2-fold dilution (50%)	11.6	18.1	32.8	46.0	56.5	56.5
5-fold dilution (20%)	12.3	16.8	28.8	45.2	51.0	68.7

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

Table 4: Results of the phytochemical analysis of the latex of *C. procera*

Phytochemicals	Amount
Alkaloids	+++
Saponins	++
Flavenoid	++
Tannins	+
Steroids	+++
Anthraquinone	+++
Triterpenoids	+++

Key: +: Trace amount, ++: Moderate amount, +++: Appreciable amount, *C. procera: Calotropis procera*

DISCUSSION

Various parts of *C. procera* 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 and etc. [6]. The results of the present study indicated that the latex of *C. procera* has antifungal potentials against dermatophytes. This finding agreed with that of Kuta, 2008, who reported the same tradition of using the *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 of the extracts.

The findings of this study are also in agreement with that of Halua and Vidyasagar 2012 who evaluated leaves extracts of two calotropis species (*C. gigantea* and *C. procera*) using three different solvents against dermatophytes and *Aspergillus flavus* with chloroform extract having the highest inhibition observed. Similarly, *C. procera* leaves extract was reported to have antifungal activity towards the tree dermatophytes genera: *Microsporum* spp., *Trichophyton* spp. and *Epidermophyton* spp. [15].

Furthermore, Iqbal *et al.* [3] reported the comparative efficacy of the chloroform and ethyl acetate *C. procera* leaf and latex extracts which proved active against some dermatophytes and other pathogenic fungi. However, the only dermatophytic fungi used in that study (*Microsporum boulardii*) was not inhibited by the extracts. This contrast with the present studies and could be explained by differences in the preparation of the plant products used and the methodology of the assay used to assess the efficacy of the plant latex.

Studies have reported several plant extracts to inhibit the growth of dermatophytes. Some of which include that of Alade and Irobi [16], who established that the ethanolic extract of *Acalypha wilkesian* had an antifungal effect on *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *A. flavus* and *Candida albicans*. Verástegui *et al.* [17], showed that the alcoholic extract of *Agave lecheguilla* has an antifungal effect on *Microsporum gypseum*, *Candida albicans*, and *Candida neoformans*. Chevallier[18] described the use of extract of *Ulmus campestris* (Elm tree), *Melissa ofinicalis* (Balm tree), and *Juglans duclouxiana* (Walnut tree) against various dermatomycotic infections.

The result of the phytochemical analysis of the fresh latex of *C. procera* has indicated the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone and triterpenoids. This result is similar to other reports on the leaves, stem and roots of *C. procera* in other studies [9,19,20]. Previous studies of phytochemical analysis of *C. procera* suggest the presence of biologically active compounds such as alkaloids, steroids, triterpenes; others include madaralbun, madarfluavil, caoutchouc, and calotropin, a very active poison of the digitalis type [21]. However, the chemical components responsible for the antifungal activity, and the mechanisms of action remain to be investigated. Though the

mechanism of action of the drug is not known, but antimycotics generally inhibit fungal growth by either disrupting fungal membrane permeability, inhibiting sterol synthesis, inhibiting the nucleic acid synthesis, or protein synthesis [22].

The results of the present study show that the plant latex is an effective antimycotic agent against dermatomycosis *in vitro*. This finding shows that there is an element of truth in the claim of traditional healers on the medicinal value of this plant as an antidermatophytic agent. Therefore, the use of the plant latex in treating dermatomycotic infections should be encouraged and the government shall pay more attention to our local medicinal plants and help in processing them, which will create more job opportunities and will bring about a reduction in the cost of conventional antifungal drugs.

REFERENCES

1. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge, New York: Cambridge University Press; 2006.
2. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995;8:240-59.
3. Iqbal J, Mishra RP, Allie AH. Antidermatophytic activity of angiospermic plants: A review. Asian J Pharm Clin Res 2015;8:75-80.
4. Behl PN, Luthra A. Bullous eruption with calotropis procera – a medicinal plant used in India. Indian J Dermatol Venereol Leprol 2002;68:150-1.
5. Fostel JM, Lartey PA. Emerging novel antifungal agents. Drug Discov Today 2000;5:25-32.
6. Mossa JS, Tariq M, Mohsin A, Ageel AM, al-Yahya MA, al-Said MS, *et al.* Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chin Med 1991;19:223-31.
7. Olatunde O. Preliminary study on phytochemical and antifungal properties of *Calotropis procera*. Sokoto: Usmanu Danfodiyo University; 2000.
8. Aliero BL, Umar MA, Suberu HA, Abubakar AA. Handbook of Common Plants in North Western Nigeria. 1st ed. Sokoto: Usmanu Danfodiyo University Press; 2001.
9. Hassan SW, Bilbis FL, Ladan MJ, Umar RA, Dangoggo SM, Saidu Y, *et al.* Evaluation of antifungal activity and phytochemical analysis of leaves, roots and stem barks extracts of *Calotropis procera* (*Asclepiadaceae*). Pak J Biol Sci 2006;9:2624-9.
10. Ameh IG, Okolo RU. Dermatophytosis among school children: Domestic animals as predisposing factor in Sokoto, Nigeria. Pak J Biol Sci 2004;7:1109-12.
11. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan, Nigeria: John Wiley and Sons Limited Sunshine House, Spectrum Books Ltd.; 1993.
12. Kennedy JF, Thorley M. Carbohydrate polymers. Pharmacognosy, Phytochemistry, Medicinal Plants. 2nd ed. Jean Brueton; Lavoisier Publishing, Paris; 2000. p. 428-9.
13. Taudou A. Active antifungigue des labiatae Donnees bibliographiques etuds in- vitrodetrieze huiler essentialles (intereldela microemulsion); 1990.
14. Dwivedi SK, Dubey NK. Potential use of the essential oil of *Trachyspermum ammi* against seed-borne fungi of Guar (*Cyamopsis tetragonoloba* L. (Taub.)). Mycopathologia 1993;121:101-4.
15. Goyal D, Sharma S, Mahmood A. Inhibition of dextransucrase activity in *Streptococcus mutans* by plant phenolics. Indian J Biochem Biophys 2013;50:48-53.
16. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. J Ethnopharmacol 1993;39:171-4.
17. Verástegui MA, Sánchez CA, Heredia NL, García-Alvarado JS. Antimicrobial activity of extracts of three major plants from the Chihuahuan desert. J Ethnopharmacol 1996;52:175-7.
18. Chevallier A. Encyclopedia of Medicinal Plants. London: Dorling Kindersley Publication, The University of Michigan; 1996.
19. Mainasara MM, Aliero BL, Aliero AA, Dahiru SS. Phytochemical and antibacterial properties of *Calotropis procera* (Ait) R. Br. (Sodom Apple) fruit and bark extracts. Int J Mod Bot 2011;1:8-11.
20. Kuta FA. Antifungal effects of *C. procera* stem back on *Epidermophyton flocossum* and *Trichophyton gypseum*. Afr J Biotechnol 2008;7:2116-8.
21. Puavilai S, Choonhakarn C. Drug eruptions in Bangkok: A 1-year study at Ramathibodi Hospital. Int J Dermatol 1998;37:747-51.
22. Betram GK. Basic and Clinical Pharmacology. 4th ed. New Jersey: Practice Hall International Inc.; 1984.

Source of Support: Nil, Conflict of Interest: None declared.