International Journal of Dermatology Research

ISSN Print: 2664-6471
ISSN Online: 2664-648X
Impact Factor: RJIF 5.42
IJDR 2023; 5(1): 21-25
www.dermatologyjournal.in
Received: 19-02-2023
Accepted: 27-03-2023

Muhammad Ali
Department of Microbiology, Federal University Gusau, Gusau, Nigeria

Nas FS
Department of Biological Sciences, Bayero University Kano, Kano, Nigeria

Garba KK
Department of Biological Sciences, Bayero University Kano, Kano, Nigeria

Ibrahim IS
Department of Pharmaceutical Technology, School of Technology, Kano State Polytechnics, Kano, Nigeria

Mu'azu L
Department of Biological Sciences, Federal University Gusau, Gusau, Nigeria

Corresponding Author:
Muhammad Ali
Department of Microbiology, Federal University Gusau, Gusau, Nigeria

Antifungal activity and phytochemical screening of *Calotropis procera* leaf extracts against some dermatophytes

Muhammad Ali, Nas FS, Garba KK, Ibrahim IS and Mu'azu L

DOI: https://doi.org/10.33545/26646471.2023.v5.i1a.39

Abstract
Medicinal plants play a vital role in covering the basic health needs in developing countries. The study was aimed to screen for phytochemicals and to determine the antifungal activity of *C. procera* leaf extracts against some dermatophytes. Clinical isolates of *Trichophyton rubrum, Trichophyton mentagrophytes Microsporum canis* and *Epidermophyton floccosum* were obtain from Pathology Department of Murtala Muhammad Specialist Hospital Kano. Phytochemical screening of the extract was done using laboratory method while agar well diffusion method was used for determination of antifungal activity of the extracts**.** The result indicated the presence of alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar, resin and terpenoid in ethanol extract while aqueous extracts contain all except resin and steroids. Based on the activity of the extracts, the finding indicated *Microsporum canis* is the most sensitive isolate with average zone of inhibition of 10.75 mm, followed by *T. rubrum* (9.50 mm), and *T. mentagrophytes* (9.00 mm) while the sensitivity was shown by *E. floccosum* with average of 8.00 mm. The ethanolic extract is more active with zone of inhibition (9.31 mm) as compared to aqueous extract (9.17 mm). It is concluded that the leaf extracts of *C. procera* possesses antifungal activity.

Keywords: Antifungal activity, *Calotropis procera*, dermatophytes, extracts

Introduction
Recently, attention has been directed towards extracts and biological compounds isolated from medicinal plants. More so, the use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer new sources of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [1]. 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 [2]. 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 [3].

The plant *Calotropis procera* (Sodom apple) belongs to the family Apocynaceae. It is found distributed all over Africa, North Africa, and Southern Sahara, from Senegal to Central Africa Republic. All the parts such as root, stem, leaf and flowers of *C. procera* are in common used in indigenous system of medicine [4]. The whole plant is toxic, with latex having strongest effect when used against cough and ringworm [5]. Human skin, the outer covering of the body, is the largest organ in the body. It also constitutes the first line of defense. Skin disease is a common ailment and it affects all ages from the neonate to the elderly and cause harm in number of ways. The skin diseases can be categorized into nine common types: rashes, viral infections, bacterial infections, fungal infections, parasitic infections, pigmentation disorders, tumors and cancers, trauma and other conditions such as wrinkles, rosacea, spider veins and varicose veins which cannot be neatly categorized [6]. Several medicinal plants possessed a wide range of dermatological effects included antibacterial, antifungal, antiviral, ant parasitic, anticancer, hair growth-promoting activity, wound and burn healing effects, for the treatment of eczema, acne, vitiligo, and psoriasis, as skin lightening, as skin protection therapy and to slow down skin ageing [7].

All parts of plant exude milky latex when cut or broken, which act as a defense strategy against insects, viruses and fungi. A large number of secondary metabolites have been isolated from this plant that includes flavonoids, cardiac glycosides, Triterpenes and sterols [8]. *Calotropis procera* is a well-known plant and has been traditionally used for the treatment of a wide range of infections globally [9]. The antimicrobial activity of *C. procera* plant extracts against bacteria and fungi is well documented [10]. Pharmacological studies of *Calotropis* species showed anti-inflammatory, anti-tumoral, antioxidant [11], antibacterial and antifungal activity [12]. The study was aimed to screen for phytochemicals and to determine the antifungal activity of in the leaf C. *procera* leaf extracts against some dermatophytes.

Material and Methods

Sampling site

The sampling site for the study is Murtala Muhammad Specialist Hospital in Kano, Kano State Nigeria. On map it is located at latitude 11^0 58' and longitude 8^0 28'. The area fall within tropical dry climate coded as AW by Koppel's. The climate is marked by distinct wet and dry seasons with flat topography and soil surface is well drained. The rainfall last for about 4-5 month annually with moderate rainfall between 370-750mm. Temperature is high with annual mean of about 22-28 °C, although climate change are believe to occur [13]. Kano State shares borders with Kaduna State to the West, Bauchi State to the South, Jigawa State to the East and Katsina State to the North. It has a total area of 20,131 km2 (7,777 sqm) and population of 13,405,300 [14].

Test isolates

Clinical isolates of *Trichophyton rubrum*, *Trichophyton mentagrophytes Microsporum canis* and *Epidermophyton floccosum* were obtain from pathology department of Murtala Muhammad Specialist Hospital in Kano, Kano State. The isolates were transported to Laboratory of Microbiology Department, Bayero University Kano for further use.

Collection and Identification of Plant Materials

The leaves of *C. procera* were collected at Rano town, Rano Local Government Area in Kano state, Nigeria at about 6:00 am. The identification and authentication of the plant materials was done at Herbarium in the Department of Plant Science Bayero University Kano with the following voucher number BUKHAN 0640. Voucher specimens were deposited there for future reference. The leaves were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into pieces and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use [15].

Fig 1: C. *procera* leaf and flower

Extraction of plant material

Aqueous (water) and ethanol solvents were used for extraction of the active components of the C. *procera* leaf. For aqueous extraction, water extraction method as described by Nas *et al*. [16] was used. 50 g of each of the grounded leaves were extracted by successive soaking for 3 days using 500 ml of distilled water in a sterile conical flask. The extracts were filtered using Whatman filter paper and the filtrates concentrated in water bath at 50 °C. The solid concentrated filtrate, now the extracts were then stored in universal bottles in the refrigerator at 4 °C before use. For ethanol extraction, 50 g of the powdered leaf was extracted in 500 ml of ethanol for 3 days mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator at 40 °C. The solid residues obtained were reconstituted in 10% DMSO at stock concentration, stored in the refrigerator at 4 ^{0}C until used.

Phytochemical Screening

Phytochemical screening of the extract was done using laboratory method to determine the presence of bioactive component present in the leaf extract of C. *procera.* Presence of Alkaloid, saponin, Glycoside, reducing Tannin, flavonoids, resin, steroid, terpenoid and Anthraquinones

were determined using procedure described by Trease and Evans [17].

Antifungal Activity of the Extracts

Agar well diffusion method was used for antifungal activity screening as preliminary test for evaluating potential activity of the leaf extracts of *Calotropis procera* [18]. Sterile Sabouraud dextrose agar (SDA) was prepared and distributed uniformly into sterile Petri plates, where 1 mL of the fungal suspension was inoculated. A sterile Cork borer 6mm was used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 50, 100, 150 and 200 mg/ml while the 5th well contained 50mg/ml Ketoconazole which served as control. The plates were incubated at room temperature for 3 days. At the end of the incubation period, fungal growth inhibition zone diameter was measure and expressed in millimeters [19].

Ethical Approval

Ethical approval for the research (issue number NHREC/17/03/2018) was obtained from Health Service Management Board Kano and Murtala Mohammed Specialists Hospital (MMSH), Kano based on the consent of the Hospitals Ethical Committees.

Results

Phytochemical Screening of C. *procera* leaf

The qualitative phytochemical screening of C. *procera* leaf aqueous and ethanolic extract is presented in Table 1. The result indicated the presence of alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar, resin and terpenoid in ethanol extract while aqueous extracts contain all except resin and steroids.

Table 1: Phytochemical Screening of *C. procera* leaf extract

S. No.	Phytochemicals	Aqueous extract	Ethanol extract
1	Alkaloid	+	+
2	Saponin	+	+
3	Phenol	+	+
4	Flavonoid	+	+
5	Glycoside	+	+
6	Tannin	+	+
7	Reducing sugar	+	+
8	Steroid	-	+
9	Terpenoid	+	+
10	Resin	-	+

Key: + = Presence of phytochemical, - = Absence of phytochemical.

Antifungal Activity of the Extracts

Aqueous Leaf Extract

The antifungal activity of aqueous extracts of C. *procera* leaf against some dermatophytes fungi is presented in Table 2. The result showed that the extract was inactive against the isolates at 50mg/ml and the activity of the extract is dose dependent with 200 mg/ml showing higher activity. From the result, *Microsporum canis* is the most sensitive isolate with average zone of inhibition of 11.0mm, followed by *T. rubrum* (9.34mm), and *T. mentagrophytes* (8.34mm) while the sensitivity was shown by *E. floccosum* with average of 8.00mm. The zone of inhibition recorded by the control (50 mg/ml Ketoconazole) was 19 mm.

Table 2: Antibacterial activity of *C. procera* aqueous leaf Extract

Concentration (mg/ml)/Zone of inhibition (mm)					
Isolates	**50**	**100**	**150**	**200**	**Control**
Trichophyton rubrum	0	6	10	12	20
Trichophyton mentagrophytes	0	6	8	11	18
Microsporum canis	0	8	12	13	20
Epidermophyton floccosum	0	0	6	8	18

Ethanol Leaf Extract

The antifungal activity of ethanol extracts of *C. procera* leaf against some dermatophytic fungi is presented in Table 3. The result showed that the extract was active against the isolates at all concentration tested and the activity of the extract is dose dependent with 200 mg/ml showing higher activity. From the result, *Microsporum canis* is the most sensitive isolate with average zone of inhibition of 10.75 mm, followed by *T. rubrum* (9.50 mm), and *T. mentagrophytes* (9.00 mm) while the sensitivity was shown by *E. floccosum* with average of 8.00 mm. The zone of inhibition recorded by the control (50 mg/ml Ketoconazole) was 19 mm.

Table 3: Antibacterial activity of *C. procera* ethanol leaf Extract

Concentration (mg/ml)/Zone of inhibition (mm)					
Isolates	**50**	**100**	**150**	**200**	**Control**
Trichophyton rubrum	6	8	11	13	20
Trichophyton mentagrophytess	6	8	10	12	18
Microsporum canis	7	10	12	14	20
Epidermophyton floccosum	6	8	8	10	18

Discussion

Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man and it can help to meet expensive and limited supply of synthetic chemicals [20]. The present study aimed to screen for phytochemicals and to determine the antifungal activity of in the leaf *C. procera* leaf extracts against some dermatophytes. Finding of this study showed that both ethanol and aqueous extracts of *C. procera* leaf contain several phytochemicals such as alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar, resin and terpenoid. Phytochemicals are naturally occurring compounds in the medicinal plants. Phytochemicals which possesses many ecological and physiological roles are widely distributed as plant constituent [21]. Plants can synthesize and accumulate in their cell a great variety of phytochemicals [22]. The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [23].

The results antifungal activity of the extracts obtained showed that both the aqueous and ethanol leaf of *C. procera* have fungicidal effects on test organisms. The results shown indicated that, ethanol was the best solvent for extracting bioactive components from this plant as compared to aqueous. The ethanolic extracts showed inhibition of growth in the test fungi with the widest zone of inhibition (9.31 mm) as compared to aqueous extract (9.17 mm). The better efficacy of the ethanol extract as against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phyto-constituents [24]. The antifungal activities of the extracts are

expected due to the presence of compounds such as alkaloid, flavonoids, saponin and tannin. The results obtained in this study corroborate with the report of Goyal *et al*. [25]; Kuta [26] and Kareem *et al*. [27] who found antifungal activity of *C. procera* leaf extracts. Based on the activity of the extracts, the finding indicated *Microsporum canis* is the most sensitive isolate with average zone of inhibition of 10.75 mm, followed by T. *rubrum* (9.50 mm), and *T. mentagrophytes* (9.00 mm) while the sensitivity was shown by *E. floccosum* with average of 8.00 mm. this finding justify the finding of Goyal and Mathur [3] who found that extracts of C. *procera* have antimicrobial potency. Komathi *et al*. [28] confirmed the antifungal potency of ethanolic extract of leaves of C. *procera*. In an investigation performed by Vadlapudi *et al.* [29] antimicrobial activity was reported by methanolic extract of aerial parts of C. *procera*.

Conclusion

From the present, it is concluded that the leaf extracts of C. *procera* contains several bioactive components alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar, resin and terpenoid which possesses antifungal activity against some dermatophytes such as *Trichophyton rubrum, Trichophyton mentagrophytes Microsporum canis* and *Epidermophyton floccosum*. It is recommended that toxicological studies on the extracts must also be performed to ensure the safety of the extracts

Acknowledgement

The authors wish to acknowledge to Technical staff of pathology Departments of Murtala Muhammad Specialist Hospital Kano for provision of isolate samples. Thanks to Kano State Government through Ministry of Health for the ethical approval.

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How to Cite This Article
Ali M, Nas FS, Garba KK, Ibrahim IS, Mu'azu L. Antifungal activity and phytochemical screening of *Calotropis procera* leaf extracts against some dermatophytes. International Journal of Dermatology Research 2023; 5(1): 21-25.