Trop J Nat Prod Res, May 2023; 7(5):3034-3037 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antimicrobial Effect of *Cassia alata*Leaf Extracts on Fungal Isolates from Tinea Infections

Ofonime M. Ogba[1]*, Dorathy I. Udoh[2], Mfoniso I. Udonkang[3], Aniekan-Augusta O. Eyo[1], Stephenie E. Chukwueke[1], Ziregbe Eshemitan[1], Nseobong G. Akpan[4]

[1]*Department of Medical Bacteriology, Virology and Mycology, Faculty of Medical Laboratory Science, University of Calabar, Nigeria.*
[2]*Department of Microbiology, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria.*
[3]*Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, University of Calabar, Nigeria.*
[4]*Instutute of Biomedical Research and Innovations, College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria.*

ARTICLE INFO

Article history:
Received 03 February 2023
Revised 10 May2023
Accepted 15 May2023
Published online 01 June 2023

ABSTRACT

Dermatophytoses are caused by fungi in the genera *Microsprum*, *Trichophyton* and *Epidermophyton*. Africans use extracts of medicinal plants to treat dermatophytosis. This study aimed to determine the in vitro antifungal activity of crude leaf extract of *Cassia alata* on fungal isolates from dermatophytosis lesions. Subjects with suspected lesions of dermatophytosis were recruited for the study. Skin scrapings were obtained from lesions for microscopy and culture. Isolates were identified macroscopically and microscopically. Fresh *Cassia alata* leaves were plucked and authenticated by a Botanist. Aqueous and alcohol extraction was done using the Soxhlet extractor. Isolates were subjected to *in vitro* leave extracts antifungal testing using disc diffusion methods. A total of 50 subjects were recruited for the study comprising 28(56.0%) males and 22(44.0%) females with a male to female ratio of 2.5: 1. The prevalence of dermatophytes infection in the study was 14(28.0%). *Trichophyton rubrum* was the most encountered isolates (42.8%). Males 10(71.4%) were more infected than females 4(28.5%). The susceptibility rates of dermatophytes to anfungals range between 0 -100% with 30(78.6%) susceptibility to Griseofulvin. The aqueous extract was more effective with susceptibility rates 33.3% - 83.3% than ethanolic extract 16.7% - 50.0%. The dermatophytes were more susceptible to the 50µg/mL aqueous extract with rates between 50% - 83.3% while the range for the 50µg/mL ethanolic extract was 25.0% - 50.0%. *Trichophyton veruccosum* and *E. floccosum* were resistant to extracts. *Cassia alata* leaf extracts had antifungal activities against dermatophytes.

***Keywords*:**Dermatophytes, *Cassia alata*, extract, antifungal effect

Introduction

Dermatophytosis also known as ringworm is a disease caused by fungi and affects the skin. It is called ringworm because it causes a circular rash, shaped like a ring. The fungal organisms are referred to as Dermatophytes. They can invade the stratum corneum of the skin epidermis, nails and hair shaft. The three pathogenic genera of dermatophytes that infect man are *Microsporum*, *Trichophyton* and *Epidermophyton*.[1] Dermatophytosis is globally distributed and endemic in tropics. The fungal growth is enhanced by the warm and moist conditions in this region. In Nigeria, dermatophyte infections rank among the top five diseases seen at dermatology clinics.[2]
Risk factors include using public showers, poor hygiene, excessive sweating, contact with infected animals, over weight and low immunity.[4] Although ringworm is not associated with fatal consequences, it can be unsightly and has economic, social and psychological impacts on those affected.[5]

*Corresponding author. E mail: ofonimemark@yahoo.com
ofonimemark@unical.edu.ng
Tel: +2348035404728

Citation: Ogba OM, Udoh DI, Udonkang MI, Eyo AAO, Chukwueke SE, Eshemitan Z, Akpan NG. Antimicrobial Effect of *Cassia alata*Leaf Extracts on Fungal Isolates from Tinea Infections. Trop J Nat Prod Res. 2023; 7(5):3034-3037 http://www.doi.org/10.26538/tjnpr/v7i5.29

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Cassia alata is a plant originating from Argentina. An annual or occasionally biannual herb belonging to the Family *Fabaceae* or *Leguminosae*. It is a tropical ornamental shrub which grows throughout the low and medium altitude. It has an average height of 1 to 4m, with growth and expansion in sunlit and humid zones. The oblong leaves have 5 to 14 leaflets, petioles measuring 2 to 3 mm, caduceus bracts (2 × 3 by 1 × 2 cm), and dense flowers (20 × 50 x 3 × 4 cm). The flowers have bright yellow color with 7 stamens containing a pubertal ovary. The fruits are tetragonal pods, thick, flattened wings, brown when ripe with many diamond-shaped brown seeds. The fruits measure 10 to 16 × 1.5 cm in length.[6-7]
Cassia alata is commonly referred to as candle brush, candlestick, *Senna alata* etc.[8] It is known as "Okoneyo", by the Annang's, "Adaiyaokon" by the Ibibio's, "Ogala" by the Ibo's and "Asunwo" by the Yoruba's.[9] Various parts of *Cassia alata* have been used in disease control. In the northern region of Nigeria, decoctions of various parts of the plant are used in treatment of skin and respiratory tract infections, burns, wounds and constipation.[10-11] In the South-western region of Nigeria, leaf decoctions has been used for the treatment of abdominal pain, toothache, convulsion and as purgative.[12-13] In Egypt, the leaf decoction is used to prevent constipation.[14] *Cassia alata* is rich in polyphenols and anthraquinones. It also contains phenols, tannins, anthraquinones, saponins and flavonoids.[15]
Dermatophytosis is common in Nigeria because of poor hygiene, low socio-economic status, hot, humid and moist environments which enhances transmission. People in the rural communities use extracts of medicinal plants including *Cassia alata* for topical treatment of dermatophytosis. These treatments usually result either in short term

relief or permanent cure. The treatment of tinea infections in Nigeria is mainly with synthetic antimicrobial agents. People in the rural community apply the *Cassia alata* extract on lesions of dermatophytosis and get short or long-lasting relief. This study may throw more light on the reason for the various responses which may be due to the infecting species variation or the quality of the extract applied. The study aimed to determine the in vitro antifungal activity of crude leaf extract of *Cassia alata* on fungal isolates from dermatophytosis lesions.

Materials and Methods

Fresh leaves of *Cassia alata* were plucked from University of Calabar Botanical Garden in March, 2021. The plant was authenticated by a Botanist, Akomaye Ferdinand in the Department of Plant and Ecological Studies, University of Calabar, Nigeria, with Voucher number: Bot/Herb/UCC/104 in March, 2021 (Plate 1).

Study population and specimen processing

This study included School children in Calabar with lesions of Tinea infection. Subjects with visible signs of Tinea infections who gave consent were included in the study. Ethical approval was obtained from the Ministry of Health, Cross River State with the Ethical reference number: CRS/MH/HREC/019/Vol.V1/198. Subjects without visible signs of Tinea infections and who did not give consent were excluded from the study. Subjects who have been on antifungal drugs for the past one month were also excluded. A structured questionnaire was administered to the study subjects for demographic information. The sites of the lesions were cleaned with alcohol swab to reduce bacterial flora. Skin scrapings were obtained from the actively growing borders of the lesions using the blunt edge of scalpels into well labeled sterile envelopes and sealed. The samples were transported to the laboratory for microscopy and culture using 20% potassium Hydroxide (KOH) solution and Sabouraud Dextrose agar (SDA) plates with Chloramphenicol (0.5mg/ml) supplement. The plates were incubated for 3 to 4 weeks at room temperature and examined every other day for the presence of fungal growth. Cultures with growth were examined macroscopically and microscopically for fungal isolates. Cultures were considered negative when no dermatophyte growth was detected after the incubation period.

Isolates were examined macroscopically, paying attention to colour, pigmentation, rate of growth and texture. Fungal isolates were examined in Lactophenol cotton blue mount microscopically for structures such as microconidia, macroconidia and chlamydospores.[16-17]

Cassia alata leaf extraction

The leaves were washed and rinsed properly in tap and sterile distilled water. Five hundred grams of the shade-dried leaves was pulverized and packed. Twenty five grams of the dried leaf powder was extracted in 250mL of absolute methanol for 24 hours. The deposit in the flask was evaporated and the extract collected.

Plate 1: *Cassia alata* plant

Preparation of stock concentration of extract

Ten (10) gram of the *Cassia alata* leaf extract was dissolved in 400mL of distilled water to give an equivalent of 1g/40mL or 0.025g/mL of the extract. This is equivalent to 25mg/mL. The stock concentration of the extract was 25mg/mL. The 25mg/mL is equivalent to 25000µg/mL. The extract concentrations for the susceptibility testing on the isolates was targeted at 10µg/mL, 25µg/mL and 50µg/mL respectively for the disc contents of Griseofulvin, Fluconazole and Itraconazole which are commonly used for the treatment of dermatophytosis.[18]

Dilution of stock extract for susceptibility testing

The One in 10 dilution of this stock in distilled water contained 2500µg/mL of the extract. The desired concentrations for the susceptibility testing of the isolates were 10µg/mL, 25µg/mL and 50µg/mL, which was obtained by carrying out 1: 250, 1:100 and 1:50 dilutions of the stock extract respectively. The various dilutions of extract were incorporated into blank sterile antibiotic discs before use. The antibiotics used as controls were obtained from Bioanalyse, Turkey.

Preparation of fungal inoculum

The inoculum of the test organisms was prepared and standardized from young cultures on Sabouraud Dextrose Agar (SDA) slants.[19] The fungi was inoculated into Brain Heart Infusion Broth (BHIB) and incubated for 24 hours at 37ºC. The overnight broth culture of each isolate was diluted in the same media to a final concentration of approximately 1 x 10^8Cfu/mL an equivalence of 0.5 McFarland Standards.[20]

Extract susceptibility testing

Disc diffusion susceptibility testing of isolates was performed using 10µg/mL, 25 µg/mL and 50µg/mL extract discs. The standardized inocula suspensions was inoculated on Mueller Hinton agar plates evenly using sterile swab sticks.[21] The antifungal agents tested as controls were Griseofulvin (10µg), Fluconazole (25µg) and Itraconazole (50 µg) by Bioanalyse (Ankara, Turkey). The susceptibility plates were incubated at room temperature for 2-5 days and examined daily for growth. The zones of inhibition were measured using a meter rule calibrated in millimeters. The measurements were compared with the CLSI standards.[22]

Statistical analysis

Statistical analysis was done with the Statistical Package for Social Sciences (SPSS) version 20.0 software (IBM Corp, Armonk, NY, USA). Frequencies and means were generated for categorical and continuous variables respectively. Interaction between specific categorical clinical variables was tested for significance using Chi square test. A *p*-value of ≤ 0.05 was considered statistically significant.

Results and Discussion

A total of 50 subjects were recruited for the study comprising 22(44.0%) females and 28(56.0%) males with a female to male ratio of 1: 2.5. The prevalence of dermatophytes infection in the study was 14(28.0%).

The distribution of dermatophytes isolates in the study is shown on Table 1. *Trichophyton rubrum* was the most encountered isolates 6(42.8%) followed by *T. mentagrophytes* 4(28.5%) while *Trichophyton verrucosum* and *Epidermophyton floccosum* were the least encountered 2(14.3%) isolates respectively.

The distribution of fungal isolates by gender among subjects is shown in Figure 1. The infection was more prevalent among males 10(71.4%) than females 4(28.6%). Males were more infected with *T. rubrum* and *T.* mentagrophytes 4(14.2%) while females were more infected with with *T. rubrum* and *E. Floccosum* 2(11.1%).

Antifungal susceptibility pattern of dermatophytes in the study

The susceptibility pattern of dermatophyte species to selected antifungal agents is shown on Table 3. The antifungal agents tested

were Griseofulvin, Itraconazole and Fluconazole. The susceptibility rates for the dermatophytes range between 0 -100%. Isolates were more susceptible to Griseofulvin 30(78.6%) but least susceptible to Fluconazole 3(21.4%). Griseofulvin was most effective on *Trichophyton mentagrophytes* and *E. floccosum* with (100%) susceptibility rates. Itraconazole was most effective on *Trichophyton mentagrophytes* (50.0%) followed by *T. rubrum* (33.3%). However, *Epidermophyton floccosum* and *T. verrucosum* isolates were not susceptible to Fluconazole 0(0.0%) and Itraconazole 0(0.0%) respectively.

The susceptibility rates of dermatophytes to aqueous and ethanolic extracts of *Cassia alata* at different concentrations of 10µg/mL, 25µg/mL and 50µg/Ml is shown in Figure 2. The three concentrations were chosen because the disc contents of the antifungal agents tested were 10µg/mL for griseofulvin, 25µg/mL for fluconazole and 50µg/mL for itraconazole. The susceptibility rates ranged from 0.0% – 83.3%. The aqueous extract was more effective with susceptibility rates 33.3% - 83.3% than the methanolic extract 16.7% - 50.0%. Dermatophytes were more susceptible to the 50µg/mL aqueous extract with rates between 50% - 83.3% while the range for the 50µg/mL methanolic extract was 25.0% - 50.0%. However, *T. veruccosum* and *E. floccosum* were not susceptible to all the ethonolic extract concentrations.

Table 1: The distribution of Dermatophytes species in the study

Type of isolates	No. (%) of isolate (n = 50)
T. rubrum	6(42.8)
T. verrucosum	2(14.3)
T. mentagrophytes	4(28.5)
Epidermophyton floccosum	2(14.3)
Total	14(28.0)

Table 2: Susceptibility pattern of dermatophytes isolates to antifungal agents

Fungal isolates	No. tested	Antifungal drugs tested No. (%) of isolates susceptible		
		AGF	ITR	FLU
T. rubrum	6	4(66.7)	2(33.3)	2(33.3)
T. mentagrophytes	4	4(100.0)	2(50.0)	1(25.0)
T. verrucosum	2	2(50)	0(0.0)	0(0.0)
E. floccosum	2	2(100)	0(0.0)	0(0.0)

KEY: AGF – Griseofulvin; ITR – Itraconazole; FLU – Fluconazole

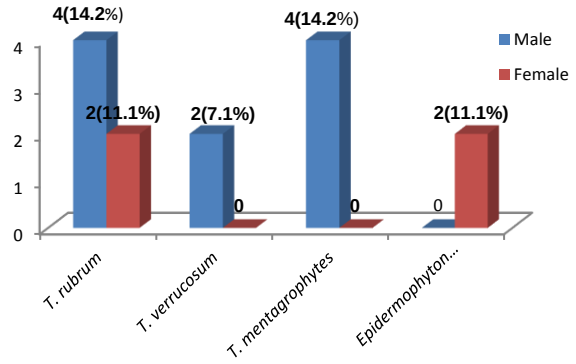

Figure 1: Distribution of Dermatophytes among subjects by gender

Dermatophytes recovery rate in the study was 28.0%. This finding differs from the work of Adefemi *et al*[23] who reported a lower dermatophytes infection rate of 5.0%. This could be due to the poor hygiene habits and lower socio-economic status of the children from the area understudy. Although the hygiene habits and sanitation was not investigated in the study, it has been observed that children are prone to dermatophytes infection because of poor environmental sanitation and personal hygiene habits.[22]

Dermatophytosis prevalence rate of 35.7% and 18.2 % respectively was recorded among male and female subjects in this study. This is higher than the 6.5% and 3.1% respectively reported by Ezihe *et al*[24] in Bauchi, Nigeria. The discrepancy may be due to the high humidity in Southern Nigeria compared to the low humidity and dry weather in the northern region of the country. The high humidity aids in the transmission of fungal skin infections.

In this study, the methanolic and aqueous leaf extracts of *Cassia alata* tested on *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton veruccosum* and *Epidermophyton floccosum* revealed a concentration dependent antifungal activity. Higher concentrations of the extracts (50µg/ml and 25 µg/ml) showed larger zones of inhibition than the lower concentrations. Aqueous extract had a high antifungal activity than methanol extract. This agrees with the reports of Abubacker *et al*[25] and Sule *et al* [26] who worked on *Cassia alata* flowers and stem bark respectively. The higher activity of the leaf extracts at higher concentrations when compared with Griseofulvin on the dermatophytes could be due to the presence of bioactive components in the extract.

The effect of aqueous leaf extract at 50µg/ml was significantly higher than the methanolic extract at the same concentration on *T. rubrum* and *T. mentagrophytes.* However, the methanolic extract concentration at 50µg/ml had no activity on *T. veruccosum* and *E. floccosum.* These findings disagrees with the work of Sule and his colleagues[26] where the crude stem bark extract of *Cassia alata* was found to inhibit the growth of *Epidemophyton floccosum* and *Trichophyton verrucosum.*

Conclusion

Cassia alata leaf extracts had antifungal activities against dermatophytes. These findings confirm the traditional therapeutic claims for these herbs to treat ringworm on the skin. This herb may serve as an alternative for the topical treatment of dermatophytosis.

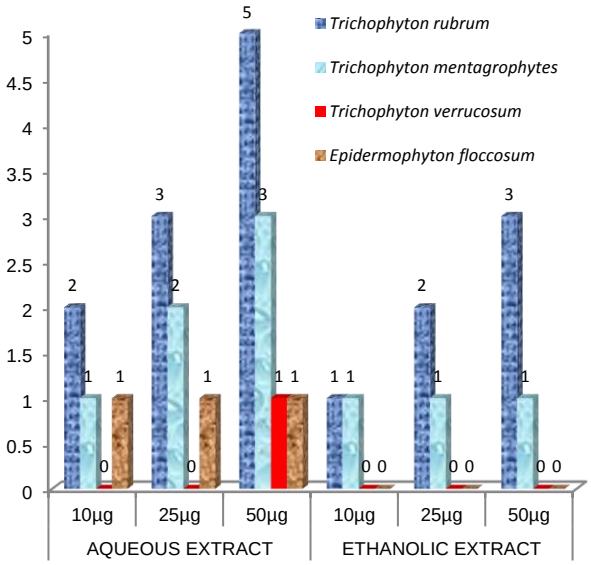

Figure 2: Susceptibility pattern of dermatophytes species to aqueous and ethanolic extracts of *Cassia alata*

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We acknowledge the management of the University of Calabar for an enabling environment for research and the University of Calabar Teaching Hospital for the use of their facility for laboratory work.

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