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In vitro Anti-dermatophyte Activities of Crude Extracts of *Cassia alata*

Chito Clare Ekwealor[1*] and Christie Amechi Oyeka[1]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka, Nigeria.

Authors' contributions

This work was carried out in collaboration with both authors. Author CAO designed the study and approved the final manuscript. Author CCE managed the analyses of the study and literature search, wrote the protocol and the first draft of the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To study the *in vitro* anti dermatophyte activities of methanol and cold water extracts of *Cassia alata.*
Study Design: Experimental.
Place and Duration of Study: Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka, Nigeria between June 2011 to January 2013.
Methodology: Clinical samples were collected from 201 rice farmers with lesions suggestive of cutaneous mycoses in Anambra State, Nigeria and identified. Methanol extraction of the dried leaves of *C. alata* and the aqueous extraction of the plant's fresh leaves were carried out. Discs impregnated with different concentrations (10mg, 20mg, 40mg, 80mg) of extracts were tested against the isolated dermatophytes. Discs impregnated with 2% Dimethyl Sulphoxide (DMSO) and 2mg/disc ketoconazole served as negative and positive controls respectively. The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of the crude extracts against the isolated dermatophytes were determined using broth dilution method.
Results: Recovered dermatophytes include *Microsporum audouinii*, *Microsporum ferrugineum*, *Trichophyton megninii*, *Trichophyton tonsurans* and *Trichophyton rubrum*. All the tested dermatophytes were sensitive to the methanol extracts of *C. alata* at concentration of ≥10mg/disc.

**Corresponding author: E-mail: ekwealorchito@yahoo.com;*

MIC value of 50mg/ml was recorded for all the test dermatophytes except *M. audouinii* whose MIC value was 25mg/ml. Fungicidal action was exhibited against the tested dermatophytes at higher concentration of ≥100mg/ml. Cold water extracts of the test plant also showed total inhibitory action against the dermatophytes.

Conclusion: The crude extracts exhibited fungicidal actions against the test dermatophytes and so the plant leaves could serve as raw materials in the development of non toxic, non resistant and affordable drugs for the treatment and control of ringworm infections.

Keywords: Dermatophytosis; crude extracts; Cassia alata; anti-dermatophyte activity.

1. INTRODUCTION

Dermatophytosis or ringworm infection is a superficial fungal infection of the skin, hair and nail. They are caused by a group of mycelial organisms known as dermatophytes that produce long chains of cells/hyphae in other to penetrate the stratum corneum and do require keratin for growth [1,2]. The distribution of dermatophytes greatly varies all around the world, with *Trichophyton rubrum* as the most common cause of the infection worldwide [3,4]. Infections may be acquired through human spread, animal to man, soil to human or indirectly by formites.

Traditional medicine practice has been known for centuries in many parts of the world for the treatment of various ailments. Though the recovery is slow, the therapeutic use of medicinal plants is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms. In Eastern Nigeria, wide variety of plants/natural products are used in the treatment of infections among which is *Cassia alata* (*Leguminosa*), which is topically applied as antimicrobial agents in the treatment of skin infections [5,6].

C. alata widely considered a weed is an ornamental shrub that grows well in forest areas of West Africa [5]. It grows in areas where there is high water table and prefers open areas and sunlight often forming thickets. The attractive shrub is named for its flower buds which grow in a column and look like fat yellow candles each complete with a flame thus giving it common names like candle stick, candle bush and Christmas candle [7,8]. *C. alata* is very effective in treating ringworm, uterine disorders, bite of poisonous snakes and also used as an anti-helminthes [9].

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Leaves of *C. alata* used in this study were collected from Anambra State in Eastern Nigeria and identified in the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. The leaves were washed under running tap, dried overnight in an oven at 40℃, grinded into fine powder and stored in an airtight container for further use.

2.1.1 Extraction of crude extracts

Thirty grams of powdered dried leaves of *C. alata* was extracted using 300ml Methanol (BDH) by Soxhlet procedure described by Horowitz [10]. Crude extracts were recovered from the solvent using rotavapour apparatus. The extracts were dried and stored in a freezer until required for analysis.

Fresh leaf (20g) was chopped and blended with 10ml of water in a Moulinex blender for 5min and the suspension filtered through muslin cloth. A clear filtrate obtained by further filtration of the suspension through Whatman No 1 filter paper and sterilized at 121℃ for 15min was used to impregnate discs (6mm) [11].

2.2 Collection, Isolation and Characterization of Dermatophytes

Two hundred and one samples from skin scrapings, hair, nails and toe web were collected from clinically suspected rice farmers from Anambra State, Nigeria. Methods of sample collection, isolation and identification are as described by Ekwealor and Oyeka [12].

2.3 Inoculum Preparation / Anti Dermatophyte Screening

Fungal inoculums were prepared according to the method of Espinel Ingroff et al. [13]. Four day old dermatophyte culture grown on SDA plates was aseptically scraped and transferred into bijou bottle containing 10ml of sterile water. The suspension was vigorously shaken, diluted Ten - fold and used for anti dermatophyte screening.

The fungal activities of the crude plant extracts against the isolated dermatophytes were investigated using disc diffusion method as described by Duraipandiyan and Ignacimuthu [14]. Sterilized discs (6mm) were impregnated with different concentrations (10mg, 20mg, 40mg, 80mg) of methanol extracts dissolved in 2% DMSO and placed on SDA plates spread inoculated with 0.1ml of 10^{-4} dilution of the inoculum preparation. The plates were prepared in duplicates, incubated at room temperature for 7 days and average diameter zone of inhibition recorded. Discs impregnated with 2% Dimethyl Sulphoxide (DMSO) and 2mg/disc ketoconazole served as negative and positive controls respectively.

2.3.1 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

MIC of the methanol extract was determined by broth dilution method [15]. Two hundred milligram of the extract dissolved in 1ml of 2% DMSO was serially diluted Two-fold in sterile water. 0.1ml of standardized suspension of the test dermatophyte (10^{-4}) was added separately to each tube containing various concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml) of the extract. This was done in duplicate and the broth medium containing no extract was used as control. The tubes were incubated at room temperature for 7days and observed for visible growth. MIC was recorded as the tube with the lowest concentration of extract that failed to show any visible macroscopic growth.

For determining the MFC, a loopful from tubes of MIC without visible macroscopic growth were inoculated on sterile SDA plates without drug supplement. The plates were incubated for 7 days at room temperature and observed for growth. The lowest concentration of the broth dilution that showed no visible growth on SDA plate was considered as the MFC [16].

3. RESULTS AND DISCUSSION

Five species of dermatophytes recovered include *Microsporum audouinii, Microsporum ferrugineum*, *Trichophyton megninii*, *Trichophyton tonsurans* and *Trichophyton rubrum*. Table 1 shows the result of the anti dermatophyte activity of crude methanol extract of *C. alata with* average diameter zone of inhibition (ZOI). Presented on Table 2, is the average zone of inhibition (mm) of cold water extract of *C. alata* while shown in Table 3 is the minimum inhibitory concentration and the minimum fungicidal concentration of methanol extract of *C. alata.*

In this study, the results obtained showed concentration dependent activity. As shown in Table 1, all the tested dermatophytes were sensitive to methanol extract of *C. alata* at concentrations of ≥10mg/disc, with *T. rubrum* showing the highest zone of inhibition (14.4mm) at concentration of 80mg/disc. Cold water extract inhibited the growth of all the isolates (Table 2).

Table 1. Anti dermatophyte activity of methanol extract of *C. alata*

Dermatophytes	Diameter zone of inhibition (mm)			
	10	20	40	80 (mg/disc)
M. audouinii	8.2	10.8	12.0	12.6
M. ferrugineum	-	12.0	12.8	13.3
T. megninii	-	9.6	10.2	11.2
T. tonsurans	-	8.2	10.4	11.0
T. rubrum	-	12.4	12.8	14.4

-= No inhibitory action

Table 2. Anti dermatophyte activity of cold water extract of *C. alata*

Dermatophytes	Diameter zone of inhibition (mm)
M. audouinii	6.4
M. ferrugineum	7.2
T. megninii	6.4
T. tonsurans	8.2
T. rubrum	8.2

Fungicidal action was exhibited against the tested dermatophytes at higher concentration of ≥100mg/ml (Table 3).

Many researchers [17,18,19] supported the results of crude methanol extract of *C. alata* as obtained in this study to be effective against dermatophytes.

In this study, MIC value of 50mg/ml was recorded for all the test dermatophytes except *M. audouinii* with MIC value of 25mg/ml. This result is contrary to the works of Alalor et al. [17] and Phongpaichit et al. [19], who reported lower MIC values of 0.625mg/ml (*T. mentagrophytes*) and 10mg/ml (*T. rubrum*) respectively. While Reezel et al. [9], recorded higher MIC values of 125mg/ml against *Trichophyton* sp. and *Microsporum gypseum*, and 62.5mg/ml for *M.*

canis, Owoyale et al. [5] reported a low MIC of 25mg/ml for *M. canis* although the leaves were extracted with ethanol.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of methanol extract of *C. alata* against the tested dermatophytes

Dermatophytes	Concentration (mg/ml)	
	MIC	MFC
M. audouinii	25	100
M. ferrugineum	50	100
T. megninii	50	200
T. tonsurans	50	200
T. rubrum	50	100

The water extract as obtained in this work inhibited all the test dermatophytes. This observation agrees with the work of Fuzellier et al. [20] and Makinde et al. [21], but differs with the report of Reezel et al. [9] and Somchit et al. [18], who noted resistance of dermatophytes to cold water extract of *C. alata*. The difference in MIC values observed in this study and those of other workers could be as a result of the difference in methods of preparation of the plant extracts and the inoculums size against which the plant extract was tested.

As presented in Tables 1 and 2, methanol and aqueous extracts of C. *alata* were observed to have good anti dermatophyte activity. Suleiman and Abdallah [22], noted a similar anti dermatophyte activity with aqueous extract of garlic, but in contrast reported that methanol extract had no effect on the test dermatophytes. This difference in methanol extract activity may have been as a result of the difference in bioactive make up of the medicinal plants.

The result of anti dermatophyte activity of *C. alata* obtained in this work supports the claims by traditional healers in Eastern Nigeria in the use of this plant for the treatment of ringworm infections.

4. CONCLUSION

The results of anti dermatophyte activities of crude extracts of *C. alata* obtained in this study was concentration dependent even though the methanol extracts showed a higher zone of inhibition values than the water extract. These leaves are inexpensive and readily available as shrubs and so could be used as raw materials in the production of non-toxic, non-resistant and affordable anti dermatophyte drugs.

CONSENT

Not applicable.

ETHICAL APPROVAL

Authors obtained oral permission from the traditional rulers of the communities where samples were collected.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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