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Assessment of Antifungal Potential of Aqueous and Methanol Extracts of *Cassia alata*

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ABSTRACT

The assessment of antifungal potential of the aqueous and methanol extracts of sun-dried leaves of *Cassia alata* was investigated by testing the extracts against some fungi (*Candida albicans, Trichophyton mentagrophyte, Aspergillus niger* and *Penicillium*) using the Agar cup plate method. The Minimum Inhibitory Concentration (MIC) of the aqueous extracts against susceptible test organism was determined using the Agar dilution method. The results showed that the plant part can be used to treat infections caused by fungi (*Candida albicans, Trichophyton mentagrophyte* and *Aspergillus niger*) which were susceptible. However, results showed that *C. alata* extract has no effect on *Penicillium*. The *in vitro* findings justify the use of the extract of *Cassia alata* in traditional medicine practice for the treatment of some external skin and fungal infections. *Cassia alata* has shown to be a very versatile plant and can be a viable alternative as an antifungal agent.

Key words: *Cassia alata*, methanol and aqueous extract, antifungal properties, *Candida albicans*, *Trichophyton mentagrophyte, Aspergillus niger*, *Penicillium*

INTRODUCTION

The use of herbal medicine predates the introduction of antibiotics and predates social, economic and religious barriers (Akinyemi *et al*., 2000). In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases (Alam, 2009). Since, ancient time, plant and animal products have been used for treatment of diseases and disorders. Plants in particular have been used to treat infections due to its antimicrobial properties (Ekachai *et al*., 2007). Plants have been classified as an essential source of medicinal agents for centuries and a huge number of novel drug components have been isolated from natural plant source and their extract used for in traditional medicine (Obeidat *et al*., 2012). *C. alata* is a pan tropical shrub which has been reported to have medicinal activities like laxative effect and active against ringworm, skin diseases and ulcer (Adnan *et al*., 2011). Ogunti and Elujobi (1993) reported that the leaves of *C. alata* are useful in treating convulsion, gonorrhea, heart failure, abdominal pains, oedema and are also used as a purgative.

Awal *et al*. (2004) observed that ethanol extract of *C. alata* has cytotoxic effect against *Artemia* and postulated that *C. alata* can be used for the treatment of cancer cell line in humans. It is locally

used in Nigeria in the treatment of several infections which include ringworm, parasitic skin diseases (Palanichamy and Nagarajan, 1990).

C. alata is one of the most important species of the genus *Cassia* which is rich in anthraquinones and polyphenols, preliminary phytochemical analysis of *C. alata* has shown the presence of phenols, tannins, anthraquinones, saponins and flavonoids (Idu *et al*., 2007).

Sharma *et al*. (2010) also reported in their study that preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone glycosides, phenolic compounds; saponin glycoside and while aqueous extract showed presence of glycosides and phenolic compounds, saponin glycoside. The leaves of *C. alata* have been qualitatively analyzed for the presence of primarily five pharmacologically active anthraquinones: rhein, aloe-emodin, chrysophanol, emodin and physcion as well as the flavonoid, kaempferol (El-Mahmood and Doughari, 2008; Moriyama *et al*., 2001). The flavonoid, kaempferol has been reported to have anticancer properties (Fernand *et al*., 2008). These anthraquinone derivatives are well known to exhibit a variety of biological activities such as antimicrobial, antifungal, antitumor, antioxidant, cytotoxic and hypoglycaemic activities.

Pieme *et al*. (2006) observed that the acute and sub-acute toxicities of hydro-ethanolic extract of leaves of *C. alata* on Swiss mice and Wistar albino rat, showed a strong evidence of the nontoxic effect of the hydroethanolic extract of *C. alata*. They also postulated that some protective effect on hepatocytes improved liver architecture. These results showed that the use of the extract of *C. alata* is safe and explained the extensive utilisation of the plant in traditional medicine. They further stated that the leaves are pounded and rubbed on the skin to cure eczema and ringworm. In treatment for ringworm, usually, the leaves are crushed and made into paste which is then spread upon the affected area of the skin. For treatment of eczema, the infected surface of the skin is washed repeatedly with strong decoction of the leaves and flowers.

Fernand *et al*. (2008) observed in their study that methanol extract of *Cassia tora* had strong antioxidant properties and that the results they obtained provided a support for the use of this plant in traditional medicine while Oladunmoye *et al*. (2007) observed in their study that ethanol extract of *Cassia hirsuta* inhibited the activities of some microorganisms by altering their genome. They further stated that the extract can be mutagenic and also possess antimicrobial activities against pathogenic bacteria.

C. alata has been proven to be effective against *C. albicans* growth culture by using the ethanol and aqueous barks extracts. Miconazole when compared to the *C. alata* barks aqueous and ethanol extract on *C. albicans* growth, showed only a slight differences between them, 30 mg mL^{-1} Miconazole with 18 mm inhibition zone and 30 mg mL^{-1} of barks aqueous and ethanol extracts with inhibition of 16 and 14 mm. This proved that this plant has potential to be exploited as a natural source of antifungal remedy in the future. With an increase of discs concentration, the extracts might produce at least the same or better effects than Miconazole (Reezal *et al*., 2002).

Pieme *et al*. (2008) observed in their study that the ethanol-aqueous extract of *S. alata* showed moderate antibacterial and antifungal activity while Krishnan *et al*. (2010) observed that acetone and Methanol extracts of *C. septabilis* were very effective on *C. albicans* more than the extracts of low polarity solvent.

Abubacker *et al*. (2008), stated that aqueous extract of *C. alata* can be used as potential antifungal agent. They observed that the aqueous extract of *C. alata* had effect on *A. flavus*, *A. parasiticus*, *F. oxysporum* and *C. albicans*. Odunbaku and Lusanya (2011) observed that the

ethanol extracts of *C. alata* leaves exhibited high activity against dermatophytic fungi, hence supporting the use of the plant in treating dermatophytic diseases caused by *Rhizopus* spp., *P. oxalicum*, *A. tamari*, *A. niger*, *F. oxysporum* and *F. vacitilus*.

A study in Malaysia (Ibrahim and Osman, 1995) reported that ethanolic extract of the *Senna* plant showed high activity against dermatophytic fungi: *T. mentagrophytes* var. *interdigitale*, *T. mentagrophytes* var. *mentagrophytes*, *T. rubrum* and *M. gypseum* (MIC: 125 mg mL^{-1}) and *Microsporium canis* (MIC: 25 mg mL^{-1}). Several studies (Akinsinde and Olukoya, 1995; Akinyemi *et al*., 2000) have been conducted to provide scientific basis for the efficacy of plants used in herbal medicine. In this study the aqueous and methanol extracts of the leaves of *C. alata* were investigated for antifungal activity.

MATERIALS AND METHODS

Plant materials: *C. alata* leaves were collected from the premises of the Federal Government College in Warri, Delta State-Nigeria and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos. The collected leaves were cleaned of unwanted foreign materials, cut up into small pieces and dried in sunlight for a week, ground and weighed. The dried material was coarsely milled, packed into a brown paper bag and stored at room temperature in the laboratory until used.

Chemicals and reagents: Sterile petri dishes, Sabouraud Dextrose agar (SDA) were purchased in Lagos. Cup borer, diameter 6 mm, Hot air oven, Cooling incubator C1-10S (REMI Instruments Ltd., Mumbai, India), Incubator (Astell Hearson, England), Uniscope SM801A Laboratory Water bath (Surgifield medicals, England), Vertical Heating pressure steam sterilizer LDZX-30FB (Labnet international Inc., Woodbridge, USA), Mettler P1210 balance (Gallen Horup) was provided by the Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos.

Extraction of plant materials

Methanol extraction: The leave material was soaked in a Winchester bottle with methanol for 48 h (maceration). The extract was concentrated using a Buchi V-801 rotary evaporator at 35°C.

Aqueous extraction: The leave material was boiled with water in a 2000 mL (2 L) of pyrex beaker at 60°C in a water bath for an hour twice, filtered and concentrated on a water bath at 70°C. The coarsely milled leaves and stem bark of *C. alata* were extracted separately using water and methanol as solvents. About 140 g of the powdered sample was continuously extracted with a particular solvent by use of a Soxhlet extraction apparatus for 24 h. The extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature (50-55°C) to obtain solvent-free semisolid extracts. The solvent-free semisolid extracts obtained were used for the antimicrobial studies.

Test microorganism and growth media: The microorganisms *C. albicans*, *T. mentagrophyte*, *A. niger* and *Penicillium* used for the study were obtained from the stocks of the Pharmaceutical Microbiology laboratory of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos.

Assay of organism: The fungal strains were grown and maintained on Sabouraud Dextrose Agar (SDA) at 30°C. With the use of SDA, all spore formers among the fungi were cultivated and

incubated at room temperature until spores developed. The spores were harvested and suspended in 1% tween 80. The turbidity was adjusted to about 10^8 SFU mL^{-1} using the serial dilution and plate count method. Candida was cultivated in SDA until growth was seen. Candida was harvested and suspended in sterile normal saline and then turbidity was adjusted to about 10^8 CFU mL^{-1}.

All standardized assay organisms were kept for the assay.

Antifungal activity evaluation: The antifungal activity of aqueous and methanol extracts of the leaves of *C. alata* at concentrations of 50, 100, 150 and 200 mg mL^{-1} were determined using the cup plate method. A molten Sabouraud Dextrose agar also stabilized at 45°C was seeded with 0.1 mL of test organism (*C. albicans*, *T. mentagrophytes*, *A. niger* and *Penicillium)* containing approximately 10^8 SFU mL^{-1} in a sterile petri dish and allowed to set.

This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas, resistant strains have smaller zones of inhibition or grow up to the edge of the disc.

Statistical analysis: The experiments were run in duplicate and the mean recorded. The zones of inhibition were determined and also recorded (mean, n = 2). The different effects of methanol and aqueous extract of *C. alata* extracts on the test organisms were analyzed using ANOVA.

Minimum inhibition concentration (MIC): The Agar dilution technique was used to determine the Minimum Inhibition Concentration (MIC) of the extract of *C. alata* against the test organisms. A stock concentration of 500 mg mL^{-1} of extract was prepared by dissolving 15 g of extract in 30 mL of propylene glycol. Ten working concentration were subsequently prepared from the stock.

Ten different concentration of the extract was used for this determination ranging from 0.625-320 mg mL^{-1}. Sterile petri dishes containing varying volumes of extract and molten agar (total volume 20 mL in each petri dish) depending on the concentration of extract intended were inoculated with 0.2 mL of the test organisms previously diluted to contain approximately 10^5 SFU mL^{-1} for fungi. A plate without an extract and another without a test organism were used as controls. The plates were incubated at 30°C for 72 h and observed for growth. The experiments were conducted in duplicate. The plate with the lowest concentration of the extract which showed no growth after incubation was taken and recorded as the MIC.

RESULTS AND DISCUSSION

Crude methanol and aqueous extract of leaves and barks from *C. alata* were assessed for selected fungi (*C. albicans*, *T. mentagrophytes*, *A. niger* and *Penicillium*).

All the crude extracts inhibited the growth of *C. albicans*, *T. mentagrophytes*, *A. niger* (Table 1, 2) as indicated by the zones of inhibition but did not show any activity against *Penicillium*. This is in line with the observations of Palanichamy and Nagarajan (1990), Ibrahim and Osman (1995), Reezal *et al.* (2002), Idu *et al.* (2007), Abubacker *et al.* (2008) and Adnan *et al.* (2011). The results showed that the extracts demonstrated a concentration-dependent antifungal activity with higher concentrations of 150 and 200 mg mL^{-1} showing greater zones of inhibition than with lower concentrations of 50 and 100 mg mL^{-1}. High MIC values are indication of low activity while low MIC values are indication of high activity. In this study, *C. albicans*, *T. mentagrophytes* and *A. niger* had low MIC values thus suggesting higher activity against the corresponding organisms (Table 3). This research has shown that methanol and

Table 1: *In vitro* antifungal activity of methanol extract of *C. alata*

Fungal species	50 mg mL^{-1}	100 mg mL^{-1}	150 mg mL^{-1}	200 mg mL^{-1}
C. albicans	10.00	14.00	14.50	16.00
T. mentagrophytes	12.00	15.00	17.00	20.00
A. niger	11.50	13.50	14.00	17.00
Penicillium	0.00	0.00	0.00	0.00

Table 2: *In vitro* antifungal activity of aqueous extract of *C. alata*

Fungal species	50 mg mL^{-1}	100 mg mL^{-1}	150 mg mL^{-1}	200 mg mL^{-1}
C. albicans	12.00	16.00	18.00	22.00
T. mentagrophytes	14.00	15.00	18.00	23.00
A. niger	12.50	15.00	17.50	20.00
Penicillium	0.00	0.00	0.00	0.00

Table 3: Minimum Inhibitory Concentration (MIC) profile of *C. alata* against test (mg mL^{-1})

Microorganisms	0.625	1.25	2.50	5.00	10.00	20.00	40.00	80.00	160.00	320.00
C. albicans	+	+	+	+	+	+	+	+	-	-
T. mentagrophytes	+	+	+	+	+	+	+	-	-	-
A. niger	+	+	+	+	+	+	+	+	-	-

+: High values; -: Low values

aqueous extracts of *C. alata* is a potential antifungal agent. This result is in line with (Palanichamy and Nagarajan, 1990; Ibrahim and Osman, 1995; Reezal *et al*., 2002; Abubacker *et al*., 2008; Krishnan *et al*., 2010; Adnan *et al*., 2011).

CONCLUSION

The finding of this study provides an insight into the usage of these plants in traditional medicine for the treatment of common fungal infections. This plant can be locally sourced since it grows well in any Nigerian soil. However, the effect of this plant against a wider range of bacteria and fungi and toxicological studies of the extracts is recommended. Further work on preformulation testing, pharmaceutical dosage formulation and development, pharmacokinetics which can be tested *in vivo* for safety and efficacy in patients is ongoing.

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