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Proximate Characterization of the Methanolic Extract of *Cassia alata* Leaves

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ABSTRACT

Preliminary phytochemical and spectrophotometric analyses as well as antifungal investigation of the methanol extract of the leaves of *Cassia alata* were carried out. Phytochemical analysis revealed the presence of alkaloids, tannins, saponnins and anthraquinones. The extract had remarkable inhibiting effects on *Trychophyton mentagrophyte* and *Microsporum audouinii* but no inhibiting effect on *Candida albicans*. The control (Endix G®) however, showed remarkable inhibiting effect on these microorganisms. The minimum inhibiting concentration (MIC) and minimum fungicidal concentration (MFC) as well as the control were determined for each microorganism. The UV/visible spectra for the extract showed maximum absorbance, 3.21 at 290 nm. The IR spectra absorption bands for the extract were recorded. The spectrophotometric data point to a possible role of anthraquinone in the observed antifungal activity of the extract.

Keywords: antifungal, anthraquinone, absorbance, concentration, inhibiting, phytochemical, spectrophotometry

INTRODUCTION

The genus *Cassia* is an attractive shrub; it has flower buds which grow in a column and looks like fat yellow candle, each complete with a flame. The leaves fold together at night. It is native to the Amazon rain-forest and can be found in Peru, Brazil, France, Guyana-Suriname, Venezuela and Colombia. Due to its beauty, it has been cultivated around the world as an ornamental plant and has been naturalized in many tropical regions in the world. These include tropical Africa, tropical Asia, Australia, Mexico, and the Caribbean, Melanesia, Polynesia and Hawaii (Wee 1992; Gangwal *et al.* 2008; Pandey *et al.* 2008).

Four different species are known: *Cassia tora*, *Cassia fistula*, *Senna alata* and *C. alata*. They serve as a prolific source of useful traditional medicine for the treatment of skin diseases, stomach problems, fever, asthma, snake bite and venereal diseases (Sofowora 1982; Wee 1992; Ogunti and Elujoba 1993; De *et al*. 2009). The use of medicinal herbs in the treatment and prevention of diseases is attracting a lot attention world wide (Okogun 1985; Iwu 1986; Kim *et al.* 1994; Close and McArthur 2002; Aderogba *et al*. 2008; Hans and Bindanda 2008). This has proved practically useful in most developing countries as a means of maintaining good health. Furthermore, increasing emphasis on the exploration of medicinal plants in industrialized countries is focused on the extraction and isolation of active agents which forms the basis for the development of several new drugs and chemotherapeutics (UNESCO 1998; Duffy *et al.* 2001).

The leaves of *C. alata* have been used to prepare a decoction as purgative treatment of convulsion, gonorrhea, heart failure, abdominal pains and oedema (Owoyale *et al.* 2005). The Peruvian herbal medicine system has prepared the leaves of *C. alata* in a decoction for the treatment of acaries, herpes ulcers, ringworm and other skin conditions (Sofowora 1982; Wee 1992). In Brazil, the extracts of the leaves have been used traditionally to treat liver problems, anemia dyspepsia, menstrual problems and high fever (Palnichamy and Nagarajan 1990). The leaves are juiced and applied to the skin to treat dermatitis and taken internally to treat syphilis (Palnichamy and Nagarajan 1990; Wee 1992). In northern Nigeria, *C. alata* and *C. tura* ethanolic mixture of leaves extracts showed high activity against dermaphytic fungi when rubbed on the skin (Ibrahim and Osman 1995).

Despite the extensive use of *C. alata* in herbal medicine, reports on detailed phytochemical investigation are rather scanty. The present research investigates methanol extracts from the leaves of *C. alata* in order to determine their phytochemical constituents in order to identify the active components that may be responsible for the plant's anti-fungal activity, which has been assessed with a view to determining its potency towards inhibiting fungal growth.

MATERIALS AND METHODS

Preparation of plant materials

Fresh leaves of *C. alata* excluding the fruits and the flowers were collected within the campus of the Federal University of Technology, Owerri, Nigeria. The plant was identified and authenticated at the herbarium of the Department of Biological Sciences, Imo State University, Owerri, Nigeria. The leaves were dried to a constant weight at 50°C, ground into coarse powder and stored in an air-tight, moisture-free container.

Extraction

The powdered plant material (15 g) was defatted with 250 ml petroleum ether for 3 hrs and then extracted by maceration for 6 hrs with 250 ml methanol. The methanolic extract was concentrated to dryness using a rotary evaporator attached to a vacuum pump and stored at 4°C until use.

Spectrophotometric analysis

The concentrated crude extract was dissolved in methanol and the resulting solution analyzed using UV/Visible (Unicam model 200A) and infrared (IR) spectrophotometers. The solvent (blank) methanol was first run to standardized the equipment at 200–800 nm. The extract showed three maximum absorbances, 3.2110, 3.1051, and 2.5010 at 290.0, 300.0 and 305.0 nm, respectively. In

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Research Note

IR spectrophotometry, NaCl was used as the infra red cell while Nichrome filament helix-wound around ceramic was the radiation source. The IR spectrum of the crude extract showed O-H (aliphatic and aromatic), N-H, C-H (aliphatic), C = O, aliphatic ether, and C-H stretching absorptions at 3600–3100, 3600–3200, 3000–2900, 1660–1640, 1120–1040 and 1460–1450 cm^{-1}, respectively.

Phytochemical analysis

The crude methanolic extract was subjected to phytochemical screening for alkaloids, saponins, tannins, phlobatannis, cardiac glycosides, anthraquinones and flavoniods as described elsewhere (Aneke *et al.* 2007).

Anti-fungal activity

The isolates *Trichophyton mentagrophytes*, *Microsporum audouini* and *Candida albicans* were obtained from the Department of Microbiology, Federal Polytechnic, Nekede-Owerri, Nigeria. They were sub-cultured on nutrient agar and stored at 4°C until use.

The antifungal activity of the crude extracts of the plant was carried out using the paper disc diffusion method (Kirby-Bauer test). The paper disc was prepared as described by Cruickshank *et al.* (1975) and Ogbuile *et al.* (2004).

In brief, 50 discs were punched from filter paper using a perforator; they were each 6 mm in diameter. The discs were boiled for 30 min in water to remove the preservative. They were then dried at 60°C in a hot air oven; the discs were stored in a bottle, capped and sterilized by dry heat at 160°C for 60 min using an autoclave. 1 ml of the crude extract was added to the bottle containing 50 discs so that each disc absorbed about 0.02 ml of the crude extract. The surface of the Sabarand® (Dongkwanb Pharm, Korea) dextrose agar was then seeded with the test organism using the spread plate method as described by Ogbuile *et al.* (2004).

Three discs previously impregnated with the extract were aseptically placed on the agar surface with the fourth disc serving as control. The plates were incubated at 30°C for 48 hrs. Endix G® (Dongkwanb Pharm, Korea), containing 10 mg econazole nitrate, l mg triamcinalone acetonide and l mg gentamicin sulphate, served as the control. The test organisms used were *Trichophyton mentagrophytes*, *Microsporum audouini and Candida albicans*. The organisms were obtained from the Department of Microbiology, Federal Polytechnic, Nekede, Owerri, Nigeria. Three discs from 50 discs previously impregnated with the crude extract were aseptically placed on the agar surfaces seeded with the test organism respectively with the fourth disc serving as control.

The zones of inhibition were measured. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration were also determined as described by Akujobi *et al.* (2004).

Statistical analysis

Analysis of variance (ANOVA) was used for data analysis. Data are reported as the arithmetic mean and standard deviation. The standard error of the mean (SEM) and generalized *t*-test was used to find the significant differences between extract means.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 shows that leaves of *C. alata* are rich in phytonutrients such as tannins, anthraquinones, saponins and alkaloids.

Anthraquniones are potent cathartic agents (Aneke *et al.* 2007). Anthraquinones have been reported to show antifungal activity against dermatophytes (Palanichamy *et al.* 1990) as well as the laxative properties of anthraquinone and saponins (Ogunti and Elujoba 1993; Owoyale *et al.* 2005). This suggests that the bio-active constituents may be responsible for the traditional use of *C. alata* leaves in the treatment of ringworm infection of the hands, feet, body, nails and scalp.

Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Okwu 2004). The presence of tannins in *C. alata* strongly supports their use in healing wounds, varicose ulcers, hemorrhoids, frostbite and burns in herbal medicine (Stray 1998).

Table 1 Phytochemical composition of *Cassia alata*.

Substance tested	Present	Not present
Alkaloid	+	
Sapnnins	+	
Tannins	++	
Phlobatannins		=
Cardiac Glycoside		+
Anthraquinone	++	
Flavonoid		=

Table 2 Peak positions and wavelength intensity of extract.

Peak position	Wavelength intensity
965.03	16.300
1020.90	16.800
1415.42	10.600
1472.19	12.505
1576.19	4.503
1650.50	10.745

Some of the general characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sadipo *et al.* 2000).

Alkaloids are ranked as the most efficient therapeutically significant plant substances. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray 1998; Egereonu and Mokwe 2005). The SEM between the methanolic extract was 0.913 while that of the control was 0.483. The generalized *t*-test was used to find the significant difference between the means. *T*- test value between the methanolic and control extract is 0.37. Hence the extract was a potent lead to antifungal drugs. Testing this value at 4 degree of freedom, $P < 0.09$.

The results obtained in this study support the use of *C. alata* leaves in the preparation of various herbal decoctions as a purgative treatment of convulsion, gonorrhea, heart failure, abdominal pain and oedema (Wee 1992). It is also used in the treatment of herpes ulcer and other skin diseases (Marowina 2010).

Spectrophotometric analysis

The methanolic extract was subjected to UV/visible spectrophotometric analysis. The extract gave a maximum absorbance at $\lambda = 290$ nm, which corresponds to the absorption maxima of the ketonic functions (RCOR) and thus confirms the presence of anthraquinone.

Characteristic IR absorption spectra of the crude extract showed a sharp absorption peak between 1600 and 1640 cm^{-1}. This might be due to the presence of the carbonyl function of an aldehyde, ketone or an amide. The absence of bands around 2850 and 2750 cm^{-1}, however indicates the absence of an aldehyde. There was also no band appearance in two regions (1300-1130 cm^{-1} and 650-850 cm^{-1}), which also precludes the presence of an ester and an amide, respectively. From the forgoing, it is obvious that the IR data preclude the presence of both aldehydes and amides in the extract. Accordingly, we can infer that the observed IR band at 1660-1640 cm^{-1} is due to a ketonic carbonyl function. The detailed peak positions and wavelength intensity of the IR spectrum is shown in **Table 2**. Correlating the UV/visible and IR absorptions, it may be suggested that anthraquinone is the major constituent of the extract.

Antifungal evaluation

The antifungal activity of the methanol extract and a commercial dermatological preparation, Endix G® against some microorganisms was evaluated; the results are shown in

Table 3 Diameter of zone of inhibition (cm).

Tested organisms	Methanolic extract	SD	SEM	Control	SD	SEM
Trychophyton mentagrophyte	3.1	0.16	0.09	2.6	0.20	0.15
Microsporum audouini	2.1	0.15	0.09	3.0	0.15	0.08
Candida albicans	0.0	0.00	0.00	1.4	0.10	0.05

Sample size (15 g); SD=Standard Deviation; SEM=Standard Error of the Mean

Table 4 Minimal inhibitory and fungicidal concentration of the methanol extracts.

Tested organism	Methanolic MIC (mg/ml)	SD	SEM	Extract MFC (mg/ml)	SD	SEM
Trychophyton mentagrophyte	62.5	4.1	2.3	62.5	4.1	2.3
Microsporum audoninii	125	0.8	0.4	125	0.8	0.4
Candida albicans	0	0.0	0.0	0	0.0	0.0

Sample size (0.02 ml crude methanolic extract); SD=Standard Deviation; SEM=Standard Error of the Mean.

Table 3. The extract showed a better biological response against *Trichophyton mentagrophytes* (dandruff) than the control. However, it did not show any effect against *Candida albicans*. The MIC and MFC, as shown in **Table 4** for the methanolic extract and control against *T. mentagrophytes* and *Microsporum audouini* (eczema), were the same.

These findings confirm the traditional therapeutic claims for *C. alata*'s use in treating ringworm/dandruff and other skin diseases. The inhibitory activities of the crude extract give promise to their potential application in the ailments or diseased conditions.

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