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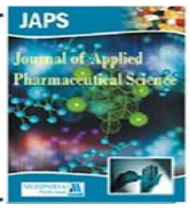

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Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of *Cassia Alata* Linn

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ABSTRACT

Cassia alata Linn is an important medicinal plant as well as ornamental flowering plant. The leaf decoction of *Cassia alata* has been used to treat infectious diseases in north eastern Nigeria. This study was embarked upon so as to evaluate the safety and efficacy of *Cassia alata* in the management of fungal infectious diseases. The leaves of the plant were collected, dried and extracted using water and 95% ethanol. The extracts were used for evaluating antifungal activity against five clinical isolates of pathogenic fungi. The result of this study showed a dose dependent antifungal activity of both aqueous and ethanolic leaf extracts on the five selected clinical isolates of pathogenic fungi. The extracts inhibited the growth of *Candida albicans, Microsporum canis* and *Trichophyton mentagrophyte* better than the ketoconazole 200 mg used as a positive control ($p<0.05$). The minimum inhibitory concentration of the water leaf extract of *Cassia alata* for *Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis* and *Trichophyton mentagrophytes* were 26.90 mg, 32.40 mg, 29.50 mg, 30.30 mg and 27.80 mg respectively, while that of ethanol leaf extract of *Cassia alata* for *Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis* and *Trichophyton mentagrophytes* were 5.60 mg, 3.50 mg, 4.90 mg, 12.60 mg and 9.80 mg respectively. *Cassia alata* has been found to exhibit a greater antifungal activity against some human pathogenic fungi in this study and this has justified the traditional use of this plant in managing fungal diseases.

Keywords: *Antifungal, Aqueous, Ethanol, Cassia alata.*

INTRODUCTION

Cassia is a native plant in Southeast Asia, Africa, Northern Australia and Latin America (Parsons and Cuthbertson, 1992) that are grown as ornamental plants (Gritsanapan and Nualkaew, 2001) with diverse medicinal uses. It is commonly known as “Rai dore” in Hausa, “Asuwon oyinbo” in Yoruba, ”Omirima” in Igbo (Arbonnier, 2004) and “Whu shil-shili” in Kilba (Timothy *et al*., 2012). The increasing development of drug resistance in human pathogens as well as the unwanted side effects of some commonly use antimicrobial agents prompted the search for newer agents with promising effectiveness and safety (Phongpaichit *et al*., 2004). Several reports have shown that *Cassia alata* contain antimicrobial substances (Fuzellier *et al*., 1982; Palanichamy and Nagarajan, 1990; Crockett *et al*., 1992; Caceres *et al*., 1993; Ibrahim and Osman, 1995; Khan *et al*., 2001; Somchit *et al*., 2003; Timothy *et al*., 2012) that may be responsible for its reported activity in bacterial and fungal infections.

Cassia alata, Cassia fistula, and *Cassia tora* are recommended for primary health care in Thailand to treat ringworm and skin diseases (Farnsworth and Bunyaprapatsara, 1992). Even though Timothy and his colleagues evaluated the antibacterial activity of the aqueous and ethanolic leaf extracts of *Cassia alata* in our environment, the antimycotic activity of the plant is yet to be evaluated despite the growing use of this plant. Therefore, this study seeks to evaluate the in vitro antifungal activity of water and ethanol leaf extracts of *Cassia alata* against some clinical isolates of pathogenic fungi.

METHODOLOGY

Source of Plant Material, Collection and Authentication

The leaf of *Cassia alata* Linn were collected in the month of September, 2011 from Hong, Hong local government area of Adamawa state, Nigeria and was identified by Mr. Mbaya of the Department of forestry and Wild life, University of Maiduguri at which the voucher specimen number (23697) was assigned and deposited in the Department.

Preparation of the Leaf Extracts

The leaves were air dried at room temperature and grounded into powder using wooden pestle and mortar. The powdered plant material was stored in an air tight container prior to extraction. The solvents used in the extraction are distilled water and ethanol.

Aqueous extract

Two hundred grams of the powdered leave were subjected to series of maceration in distilled water (200 g/1.5 L) and the extract decanted at an interval of 24 hours. The filtrate was then evaporated to dryness in an oven (ewerka oven) at 40^{o}C giving a dark green solid with a yield of 12.5 g (6.25%).

Ethanol extract

Two hundred grams of the powdered leave was subjected to maceration in ethanol (200 g/1.5 L). The extract was then concentrated to dryness under pressure giving a dark green solid with a yield of 16.4 g (8.2%).

Source of the Microorganisms

Clinical isolates of the test organisms (*Candida albican, Microsporum canis, Trichophyton mentagrophyte, Penicillium notatum* and *Aspergillus niger*) were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH).

Media preparation and the cup holes

The Sabuoraud dextrose agar (SDA) was prepared according to the manufacturer's specification. A double layer of 30 ml of the Media was prepared in a Petri dish. This prepared media was then sterilized by autoclaving at 121^{0}C for 15 minutes after which it was allowed to cool and then used for culturing the microorganisms. Holes were bored on the culture media plate using a sterile cup borer 6 mm in diameter which was labelled C1, C2, C3, C4, C5 and C6. Each cup hole contains between 25 mg, 50 mg, 100 mg and 200 mg of 500 mg/ml stock of the extracts obtained by appropriate dilutions, 80 mg of ketoconazole and 0.4 ml of distilled water respectively.

The distilled water (0.4 ml) and ketoconazole (200 mg/ml) were used as the negative and positive controls respectively.

Antimicrobial susceptibility testing of the plant extract

This was carried out using the cup-plate method where the various strengths of the extracts were put into their corresponding holes on the SDA plate containing *Candida albican, Microsporum canis, Trichophyton mentagrophyte, Penicillium notatum* and *Aspergillus niger*.

In each case, 6 different holes were sunken on the media plate in which 25 mg, 500 mg, 100 mg and 200 mg of the extract, 4 ml of distilled water and 200 mg of ketoconazole were put into the holes. The plates were allowed for an hour to diffuse and then incubated for 24 hours (*Candida albicans*) and 120 hours (*Microsporum canis, Trichophyton mentagrophyte, Penicillium notatum* and *Aspergillus niger*) after which they were examined for zones of inhibition and readings were taken in millimeters.

Determination of minimum inhibitory concentration

The MIC values of the *Cassia alata* leaf extracts on *Candida albican, Microsporum canis, Trichophyton mentagrophyte, Penicillium notatum* and *Aspergillus niger* were obtained by extrapolation from the plot of log strength (mg) of the extract against zone of inhibition (mm) (Timothy *et al*., 2011).

Statistical Analysis

Student t-test was used in the analysis to determine the level of significance of the various bacterial zones of inhibition observed. P-value less than 0.05 were considered significant.

RESULT ANALYSIS

Antifungal screening of aqueous leaf extract of *C. alata*

The result of this study showed a dose dependent antifungal activity of aqueous leaf extract of *Cassia alata* at 50 mg to 200 mg on five selected clinical isolates of pathogenic fungi.

At 100 mg and 200 mg of the extract there was a higher statistical significant difference in activity than the positive control (Ketoconazole) on *Candida albican, Microsporum canis* and *Trichophyton mentagrophyte* than the ketoconazole 200 mg used as a positive control ($p<0.05$). The effect of water extract of *Cassis alata* on *Aspergillus niger* at 100 mg did not show a statistical significant difference with the ketoconazole ($p>0.05$). However, at 200 mg the water leaf extract had a higher antifungal activity on *Aspergillus niger* than ketoconazole ($p<0.05$). There was no statistical significant difference between the effect of the water leaf extract at 100 mg and 200 mg with the positive control (ketoconazole) against *Penicillium notatum* observed in this study ($p<0.05$) (Table 1).

Table. 1: Antifungal screening of aqueous leaf extract of *Cassia alata* showing the zones of inhibition (mm) (n=5).

Organism	*+ve control*	*Mean zones of inhibition of Water extract (mm)*			
		25 mg	*50 mg*	*100 mg*	*200 mg*
C. albican	25.40±1.14	N.I	20.20±0.68*	29.80±0.60*	30.00±0.78**
A. niger	28.20±0.64	N.I	10.50±0.29**	27.80±0.54	33.80±0.84*
P. notatum	20.40±0.54	N.I	15.20±0.50*	19.80±0.52	22.00±1.08
M. canis	25.40±1.10	N.I	17.20±0.34*	29.80±0.43*	32.00±0.78**
T. mentagrophytes	20.40±0.74	N.I	20.20±0.41	26.80±0.72*	35.00±0.58**

* indicates a significant difference at p<0.05 ** indicates a significant difference at p<0.01
+ve control = Ketoconazole 200 mg, N.I = No inhibition, C. albicans = Candida albicans
A. niger = Aspergillus niger, P. notatum = Penicillium notatum, M. canis = Microsporium canis
T. mentagrophytes = Trichophyton mentagrophytes

Table. 2: Antifungal screening of ethanol leaf extract of *Cassia alata* showing the zones of inhibition (mm) (n=5).

Organism	*+ve control*	*Mean zones of inhibition of Ethanol extract (mm)*			
		25 mg	*50 mg*	*100 mg*	*200 mg*
C. albican	24.00±1.58	19.80±0.64*	26.20±0.74	29.80±0.64*	36.00±0.81**
A. niger	34.20±0.84	20.20±1.04**	17.60±1.14**	21.00±0.28**	25.80±0.40**
P. notatum	23.40±0.54	19.40±0.81*	20.20±0.83	21.80±0.34	30.00±0.68**
M. canis	20.40±1.10	14.40±0.42**	15.20±0.74	25.80±0.34*	30.00±0.48**
T. mentagrophytes	18.40±0.74	16.40±0.54*	19.20±0.53	26.80±0.24**	30.00±0.58**

* indicates a significant difference at p<0.05 ** indicates a significant difference at p<0.01
+ve control = Ketoconazole 200 mg, C. albicans = Candida albicans, A. niger = Aspergillus niger , P. notatum = Penicillium notatum, M. canis = Microsporium canis , T. mentagrophytes = Trichophyton mentagrophytes

Antifungal screening of ethanol leaf extract of *Cassia alata*

Ethanol leaf extract of *Cassia alata* at the tested doses of 25 mg to 200 mg on five selected clinical isolates of pathogenic fungi, showed a dose dependent effect. At 100 mg and 200 mg of the extract there was a higher statistical significant difference in activity than the positive control (Ketoconazole) on *Candida albican, Microsporum canis* and *Trichophyton mentagrophyte* than the ketoconazole 200 mg used as a positive control (p<0.05). The effect of ethanol extract of *Cassis alata* on *Penicillium notatum* at 100 mg did not show a statistical significant difference with the ketoconazole (p>0.05). However, at 200 mg the ethanol leaf extract had a higher antifungal activity on *Penicillium notatum* than ketoconazole (p<0.05). Ketoconazole was able to inhibit the growth of *Aspergillus niger* significantly higher than ethanol leaf extract at all the doses tested (p<0.05) (Table 2).

Table. 3: MIC for Water and Ethanol leaf Extract of *Cassia alata*

S/No.	*Organism*	*MIC (mg)*	
		Water extract	*Ethanol extract*
1	*C. albican*	26.90	5.60
2	*A. niger*	32.40	3.50
3	*P. notatum*	29.50	4.90
4	*M. canis*	30.30	12.60
5	*T. mentagrophytes*	27.80	9.80

C. albicans = Candida albicans, A. niger = Aspergillus niger,
P. notatum = Penicillium notatum, M. canis = Microsporium canis,
T. mentagrophytes = Trichophyton mentagrophytes

Minimum inhibitory concentration of aqueous and ethanol leaf extract of C*assia alata*

The minimum inhibitory concentration of the water leaf extract of *Cassia alata* for *Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis* and *Trichophyton mentagrophytes* were 26.90 mg, 32.40 mg, 29.50 mg, 30.30 mg and 27.80 mg respectively obtained from the graph by extrapolation, while that of ethanol leaf extract of *Cassia alata* for *Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis* and *Trichophyton mentagrophytes* were 5.60 mg, 3.50 mg, 4.90 mg, 12.60 mg and 9.80 mg respectively (Table-3).

DISCUSSION

The aqueous and ethanolic leaf extracts of *Cassia alata* tested on *Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis* and *Trichophyton mentagrophytes* showed a dose dependent antifungal activity. This agrees with several reports in which similar observations were made (Ibrahim and Osman, 1995; Khan *et al.*, 2001; Somchit *et al.*, 2003; Makinde *et al*., 2007; Abubacker *et al*., 2008; Sule *et al*., 2011) even though some worked on flowers (Abubacker *et al*., 2008), while others evaluated the stem bark (Sule *et al*., 2011). The higher statistical significant difference in activity of the leaf extracts at higher doses when compared with Ketoconazole on *Candida albican, Microsporum canis* and *Trichophyton mentagrophyte* (p<0.05) could be attributable to the presence of some bioactive components in the extract. However, on *Aspergillus niger* and *Penicillium notatum* the ketoconazole and the leaf extract exhibits varied antifungal activity. The susceptibility of the plant extract could be attributed to the absence of a polysaccharide Nigeran in some of these organisms which is present in the *Aspergillus niger* (Makinde *et al.,* 2007; Ogunti and Olujoba, 1993). The effect of aqueous leaf extract at 200 mg is statistically significantly higher on *Trichophyton mentagrophyte* than on *Candida albican* (p<0.05). This finding agrees with the report of Sule and his colleague in which the crude stem back extract of *Cassia alata* was found to significantly inhibit the growth of *Trichophyton verrucosum* and *Epidemophyton floccosum.*

Generally the ethanol leaf extract showed a higher growth inhibition than ketoconazole on all the organisms used in this study except on *Aspergillus niger*. The report of Makinde *et al* (2007) and Ogunti and Olujoba (1993) in which the ethanol plant extract

was found to exhibit marked antimicrobial activity against *Aspergillus niger* and *Candida albicans* when compared to water extracts partly agrees with the result of this study in which the effect of ethanol extract on *Candida albicans* was significantly higher than the water leaf extract ($p<0.05$). However, the results of the present study did not agree with the report of Makinde *et al* (2007) and Ogunti and Olujoba (1993) in which the aqueous extract at 100 mg and 200 mg was found to inhibit the growth of *Aspergillus niger* significantly higher ($p<0.05$) than the ethanol leaf extract. This study showed that the crude ethanol extract had higher antifungal activity against *Candida albicans* and *Penicillium notatum* as compared to the water extract of the same plant. The presence of anthraquinone in the ethanolic leaf extract earlier detected by Timothy *et al* (2012) which were presumably absent in the aqueous extract may be responsible for the variation in antifungal activity. Conversely, the water extract had higher activity on *Aspergillus niger* and *Trichophyton mentagrophytes* than the ethanolic leaf extract.

The MIC showing *Aspergillus niger, Penicillium notatum and Candida albicans* being more susceptible to the ethanolic leaf extract when compared with *Trichophyton mentagrophytes* and *Microsporum canis* did not agree with Bharathidasan *et al* (2011) who reported that the ethanol leaf extract had activity at lower concentration on *Candida albicans* as compared to *Aspergillus niger*. However, *Candida albicans* was more susceptible to water extract than the other organisms studied. This study amply justifies the ethno medical use of this plant in the management of dermatophytosis and other fungal diseases.

CONCLUSION

Cassia alata has been found to exhibit a greater antifungal activity against some human pathogenic fungi in this study. Therefore, further efficacy and safety studies are encouraged on this potential herb with the hope of replacing some less effective ones in clinical practice.

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