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

Timothy SY, Wazis CH, Adati RG and Maspalma ID

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 northeastern 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*, *Microsporum 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*, *Microsporum 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; Palanchamy 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 Bunyaprapsatsara, 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 antymycotic 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°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, Microsoporum canis, Trichophyton mentagrophyte, Penicillium notatum and Aspargillus 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°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, Microsoporum canis, Trichophyton mentagrophyte, Penicillium notatum and Aspargillus 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 (Microsoporum canis, Trichophyton mentagrophyte, Penicillium notatum and Aspargillus 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, Microsoporum canis, Trichophyton mentagrophyte, Penicillium notatum and Aspargillus 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, Microsoporum 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 Aspargillus 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 Aspargillus 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).

<table>
<thead>
<tr>
<th>Organism</th>
<th>+ve control</th>
<th>Mean zones of inhibition of Water extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>C. albicans</td>
<td>25.40±1.14</td>
<td>20.20±0.68*</td>
</tr>
<tr>
<td>A. niger</td>
<td>28.20±0.64</td>
<td>15.20±0.50*</td>
</tr>
<tr>
<td>P. notatum</td>
<td>20.40±0.54</td>
<td>17.20±0.34*</td>
</tr>
<tr>
<td>M. canis</td>
<td>25.40±1.10</td>
<td>20.20±0.41</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>20.40±0.74</td>
<td>26.80±0.72*</td>
</tr>
</tbody>
</table>

* indicates a significant difference at p<0.01.

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>+ve control</th>
<th>Mean zones of inhibition of Ethanol extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>C. albicans</td>
<td>24.00±1.58</td>
<td>19.80±0.64*</td>
</tr>
<tr>
<td>A. niger</td>
<td>34.20±0.84</td>
<td>20.20±1.04**</td>
</tr>
<tr>
<td>P. notatum</td>
<td>23.40±0.54</td>
<td>19.40±0.81*</td>
</tr>
<tr>
<td>M. canis</td>
<td>20.40±1.10</td>
<td>15.20±0.74</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>18.40±0.74</td>
<td>16.40±0.54*</td>
</tr>
</tbody>
</table>

* indicates a significant difference at p<0.01.

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 albicans, Microsporum canis and Trichophyton mentagrophytes than the ketoconazole 200 mg used as a positive control (p<0.05). The effect of ethanol extract of Cassia 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

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Organism</th>
<th>MIC (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C. albicans</td>
<td>26.90</td>
</tr>
<tr>
<td>2</td>
<td>A. niger</td>
<td>32.40</td>
</tr>
<tr>
<td>3</td>
<td>P. notatum</td>
<td>29.50</td>
</tr>
<tr>
<td>4</td>
<td>M. canis</td>
<td>30.30</td>
</tr>
<tr>
<td>5</td>
<td>T. mentagrophytes</td>
<td>27.80</td>
</tr>
</tbody>
</table>

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

Minimum inhibitory concentration of aqueous and ethanol leaf extract of Cassia alata

The minimum inhibitory concentration of the water leaf extract of Cassia alata for Candida albicans, Aspergillus niger, Penicillium notatum, Microsporum 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, Microsporum 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, Microsporum 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 albicans, Microsporum canis and Trichophyton mentagrophytes (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 Nigerian 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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