Antidermatophytic and antioxidant activity of hexane extracts of *Cassia alata* leaves and its phytochemical screening

J. Sujatha¹ and S. Asokan²

¹PG and Research Department of Microbiology, Marudupandiyar College, Vallam, Thanjavur, Tamil Nadu, India - 613 403.
²Department of Microbiology, Annai college of Arts and Science, Kovilacheri, Kumbakonam-612 503.

**Article info**

**Abstract**

The aim of the study was to evaluate the free radical scavenging and antidermatophytic activity of hexane extract of *Cassia alata* leaves. Hexane extract was prepared by using soxhlet apparatus by boiling 50 g of dried leaves powder mixed with 200 ml of hexane. Prepared hexane extract was screened *in-vitro* for their antioxidant and antidermatophytic activity. The inhibitory concentration (*IC₅₀*) of hexane extract against free radical DPPH was assayed. The antidermatophytic activity was performed against *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Microsporum canis*. Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the hexane extract was determined as 29.3µg/ml. The MIC value of the hexane extract was determined ranging between 20 and 75 µl/ml. The MFC values of the hexane extracts is 25 µl /mL. From these results we concluded that the extracts of *Cassia alata* leaves could be exists effective antioxidant and antidermatophytic activity even at minimum concentrations. However, hexane extracts exhibits excellent antifungal agent properties and it found to be alternative to chemotherapeutic agents.

**Key words:** *Cassia alata*, Antioxidnat, Antidermatophytic activity, Phytochemicals.

**INTRODUCTION**

The ancient medicinal system was greatly depending on the plant materials due to the presence of bioactive secondary metabolites. These are involving in the curing health related
problems and acts as protecting agents because of their complex structure [1,2]. Phytochemical screening methods of green plant extracts have been of great interest to researchers in the development of new drugs for effective treatment of life threatening several diseases [3]. Plant derived drugs from different varieties plants used to finding new and novel chemotherapeutics to treat various diseases like cancer, fever, stomach pain, inflammation and viral as well as microbial infection [4].

Dermatophytosis is the most common superficial fungal infections in India which is otherwise known as ringworm disease. The most common dermatomycoses caused by the species of *Epidermophyton, Microsporum* and *Trichophyton*. Mostly, this disease is widely treated by preferred drug like terbinafine, Griseofulvin and triazoles. Recently, the fungi are developed resistance to these chemotherapeutic drugs [5]. In which based on this statement, the improved cure rate drug with reduced side effects, lower cost has been developed to decrease the resistance of fungi against drug. In this context, solvent extracted phytochemical constituents from plants offers alternatives for the treatment of dermatophytosis. Plant derived drugs has been found favorable choice to combat dermatophytes infection or reduce the risk of side effects and these natural derived plant drugs are preferred for human wellbeing [6].

The plant *Cassia alata* Linn. was scientifically classified under the family Caesalpiniaceae. Ringworm weed is the common name and it is an annual herb with compounded leaves. This plant has been potentially used in medicinal system because of presence of wide range of bioactive molecules, developing the rich resource of different types of medicines. *Cassia alata* is one of the most important species in the genus of Cassia [7]. *C. alata* contains rich amount of anthraquinones and polyphenols [8-10]. Antimicrobial activity of the plant is associated with the presence of other phytochemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates [11]. Likewise, crushed leaves of this plant are effective in treating various skin diseases like ringworm, eczema, pruritis, itching, and scabies in humans [12-14]. Moreover, leaves were used to prepare herbal tea, herbal soaps, shampoos and skin care cosmetic products to cure dermatological skin diseases [15]. In this screening study for antidermatophytes compounds from *C. alata* was performed by evaluating activity of hexane extract of *C. alata* leaves and also study the DPPH scavenging assay.

**MATERIALS AND METHODS**

**Preparation of plant extract**

Fresh leaves were washed with water and shade dried at room temperature for 3 – 4 days. Shade dried leaves were grounded into powder sieved through mesh to obtain to get the uniform sized powder. About 50 g of leaf powder was weighed and packed with filter paper. Then the powder sample was inserted into soxhlet apparatus and the extraction was performed by using 200 ml of hexane. This process is continued for 3 hrs at the temperature 45 °C. After extraction, collect the filtrate and dried in rotary evaporator to obtain crude
extract. This crude extract used to study phytochemical screening, anticancer and antidermatophytic activities.

**Preliminary Phytochemical analysis**
The phytochemical screening of hexane crude extracts were analyzed for the presence of alkaloids, glycosides, carbohydrates, quinines, saponins, phenols, tannins, flavanoids, steroids, terpenoids, proteins and sugars compounds according to the standard procedures [16-19]. To phytochemical analysis, 10 mg of crude extract was diluted with 10 ml of distilled water.

**Analytical technique**
Gas chromatograph interfaced to a Mass spectrometer analysis of the hexane crude extract was performed using Perkin-Elmer GC Clarus 500 system. The result of obtained elution was compared with their relative retention time and MS spectra with library data. GC-MS spectrum asserts name, molecular weight and structure of the components of elution. The FTIR spectrum of hexane extract was carried out at the wave number from 4000 to 500 cm\(^{-1}\) to identify the presence of bioactive functional groups.

**Free radical scavenging assay (DPPH)**
DPPH scavenging activity of crude hexane extract was examined according to the method reported by Gyamfi et al [20] and Halliwell [21]. DPPH scavenging activity assay of hexane extract of *C. alata* leaves were performed by preparing 1 mM DPPH was dissolved in methanol. About the DPPH solution, 450 µl of 50 mM Tris-HCl buffer (pH 7.4) was mixed and the solution was covered by aluminium foil to protect DPPH solution from sunlight. A 3 ml of different concentration of hexane extract like 5, 20, 40, 60, 80 and 100 µg/ml were taken and mixed with 0.5 ml of DPPH solution and incubate in dark condition for 30 minutes. After incubation the optical intensity of the reaction mixture was examined in Spectrophotometer at 517 nm. Ascorbic acid was used a positive control and the methanol solution was considered as blank. Control was maintained without addition of leaf extract. The percentage of DPPH inhibition by hexane solvent extracts of *C. alata* leaves was calculated by following the equation:

\[
\text{% of Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

The inhibition concentration to scavenge 50% free radical (IC\(_{50}\)) is determined by plotting a graph of concentration (µg/ml) against percentage (%) of free radical inhibition.

**Anti-dermatophytic activity of hexane extracts of *C. alata* leaves**

**Collection of dermatophytes**
Dermatophytes like *Trichophyton mentagrophytes* (MTCC-7687), *Trichophyton rubrum* (MTCC-7859), *Epidermophyton floccosum* (MTCC-7880), *Microsporum audouinii* (MTCC-8197), and *Microsporum canis* (MTCC-3270) were collected from MTCC, Chandigarh.

**Determination of Minimum Inhibitory Concentration (MIC)**
The MIC of the hexane extract was determined by diluting the different concentrations (25-75 µl/ml) hexane extract. Minimum zone of inhibition of hexane crude extract against dermatophytes is denoted as minimum inhibitory concentration (MIC). In this typical assay,
0.1ml of standardized inoculums spores of $1 \times 10^7$ cfu/ml was uniformly spread on petriplates containing solidified sterilized Saboroud Dextrose Agar (SDA). After that 3 wells were made with the 6 mm diameter by using gel puncture and add different concentrations of hexane plant extract. Then the plates were incubated at $37^\circ$C for 24 to 48 hrs and measure the zone of growth inhibition around the well. The lowest concentration of extract with no visible fungal growth on solid medium was regarded as MIC. The percentage of zone of inhibition was calculated by finding difference between activity of control and sample of hexane extract. The positive control terbinafine activity against dermatophytes is considered as 100% of inhibition.

**Determination of Minimum Fungicidal concentration**

The in vitro minimum fungicidal activity (MFC) was determined described by Espinel-Ingraff et al [22, 23]. To determine the MFCs, 1 ml of standardized inoculums spores of $1 \times 10^7$ cfu/ml was mixed with Saboroud Dextrose broth and subsequently adds the different concentrations of plant extract from 10-100 µg/ml. Then all the broths were incubated in aseptic conditions at $37^\circ$C for 24 to 48 hrs. After 48 hrs 0.1 ml of inoculums was withdrawn from each concentration contained broth and inoculated on Sabouraud dextrose agar for the examination of MFC. Plates were incubated at $37^\circ$C for 72 h. MFC was defined as the lowest drug concentration that showed less than 3 colonies or no visible growth on the plates is considered as an inhibition activity of 99% or 100%, respectively.

**RESULT AND DISCUSSION**

**Phytochemical screening and antioxidant activity of C. alata leaves**

**Preliminary screening assay**

The phytochemical analysis of hexane leaf extract of *Cassia alata* was analysed for the phytocompounds such as carbohydrates, tannin, phenol, saponin, flavonoid, steroid, terpenoids, glycosides, alkaloids. Among them, glycosides, quinines, saponins, phenols, flavanoids, and steroids were present in the extract. Alkaloids, carbohydrates, tannins and terpenoids were not present in the hexane solvent extract. Moreover, among the twelve phytochemicals hexane extract has 7 compounds out of 12 (Table 1).

**Table 1: Phytochemical screening of hexane solvent extract of C. alata leaves**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>Hexane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>_</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>_</td>
</tr>
<tr>
<td>4</td>
<td>Quinines</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>_</td>
</tr>
<tr>
<td>8</td>
<td>Flavanoids</td>
<td>+</td>
</tr>
</tbody>
</table>
Heaxane extract of \textit{C. alata} leaves revealed a presence of H-bonded O-H stretching alcohols and phenols at 3245 cm\(^{-1}\). Two weak bands were observed at 2918 and 2848 cm\(^{-1}\)corresponds to presence of O-H stretching carboxylic acids and C-H stretch off C=O aldehydes, respectively. The two weak peaks at 1722 and 1602 cm\(^{-1}\) revealed to C=O stretching ketones and N-H bend primary amine, respectively. The absorption bands at 1452 and 1203 cm\(^{-1}\) were recorded which are characterized to C-C stretch aromatics and C-N stretching aliphatic amines, respectively. A strong and narrow peak was positioned at 1010 cm\(^{-1}\) corresponds to C-O stretch esters and ethers (Figure 1 and Table 2).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|p{0.7\textwidth}|}
\hline
S.No & Wave number (cm\(^{-1}\)) & Functional groups \\
\hline
1 & 3245 & H-bonded O-H stretching alcohols and phenols \\
2 & 2918 & O-H stretching carboxylic acids \\
3 & 2848 & C-H stretch off C=O aldehydes \\
4 & 1722 & C=O stretch ketones \\
5 & 1602 & N-H bend Primary amine \\
6 & 1452 & C-C stretch (in-ring) aromatics \\
7 & 1203 & C-N stretch aliphatic amines \\
8 & 1010 & C-O stretch esters and ethers \\
\hline
\end{tabular}
\caption{FTIR spectrum of hexane extract of \textit{C. alata} leaves}
\end{table}
The characterization technique of GC-MS spectrum of the hexane extract of *C. alata* leaves showed eight peaks indicate the presence of eight different phytochemical components (Figure 2). The identified chemical constituents are represented in Table 3. Hexane chromatogram shows one major peak namely Octadecanal, other minor peaks represents presence of phytol, Heptacosane, Tetratriacontane, 14-Heptadecenal, 3-Butoxy-1,1,1,5,5,5-Hexamethyl-3-(trimethylsiloxy) trisiloxane, and 9-Octadecene, 1-[3-(Octadecyloxy) propoxy]-, (Z).

![Figure 2: GC-MS chromatogram shows the presence of phyto-constituents of hexane extract of *C. alata* leaves](image)

### Table 3: GC-MS chromatogram shows the presence of phytoconstituents of hexane extract of *C. alata* leaves

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Name of the compound</th>
<th>Molecular weight</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.33</td>
<td>Phytol</td>
<td>296</td>
<td>C_{20}H_{40}O</td>
</tr>
<tr>
<td>23.27</td>
<td>Heptacosane, 1-Chloro-</td>
<td>414</td>
<td>C_{27}H_{55}Cl</td>
</tr>
<tr>
<td>24.72</td>
<td>Tetratriacontane</td>
<td>478</td>
<td>C_{34}H_{70}</td>
</tr>
<tr>
<td>25.87</td>
<td>Heptacosane, 1-Chloro-</td>
<td>414</td>
<td>C_{27}H_{55}Cl</td>
</tr>
<tr>
<td>26.95</td>
<td>14-Heptadecenal</td>
<td>252</td>
<td>C_{17}H_{32}O</td>
</tr>
<tr>
<td>28.49</td>
<td>octadecanal</td>
<td>268</td>
<td>C_{18}H_{36}O</td>
</tr>
<tr>
<td>29.81</td>
<td>3-Butoxy-1,1,1,5,5,5-Hexamethyl-3-(trimethylsiloxy) trisiloxane</td>
<td>368</td>
<td>C_{13}H_{36}O_{4}Si_{4}</td>
</tr>
<tr>
<td>30.86</td>
<td>9-Octadecene, 1-[3-(Octadecyloxy) propoxy]-, (Z)</td>
<td>578</td>
<td>C_{39}H_{78}O_{2}</td>
</tr>
</tbody>
</table>
HPLC analysis

HPLC chromatograms shown three phyto-constituents of the hexane extract of *C. alata* leaves are shown in Figure 3. It appears to contain predominantly two compounds which were eluted at a retention time (Rt) of 2.098 min and 2.554 min respectively while the minor peak was observed at a Rt of 4.300 min.

![HPLC Chromatogram](image)

**Figure 3: HPLC chromatogram characterization of hexane extract of *C. alata* leaves**

Antioxidant activity of hexane extract of *C. alata* leaves

In order to the antioxidant activity of the solvent hexane leaf extract of *C. alata* was determined by DPPH scavenging activity by measuring the optical density of colored solution using Spectrophotometer at the wavelength of 517 nm. DPPH scavenging activity of hexane extract is dependent on the dosage manner (Figure 4 &5). The 50% of inhibition concentration (IC₅₀) of hexane extract and standard was found to be 29.3µg/ml and 35.6 µg/ml, respectively (Table 4). The antioxidant activities of the hexane extract may be due to the presence of glycosides, quinines, saponins, phenols, flavonoids and steroids in the leaves. These phytochemical may responsible for biological activities such as anticancer, antioxidant, antidiabetics, antimicrobial and anti-inflammatory properties.
Figure 4: DPPH scavenging activity of *Cassia alata* hexane Extract

![Graph showing DPPH scavenging activity](image)

Figure 5: Regression analysis of antioxidant activity of hexane extract of *C. alata* leaves

![Graph showing regression analysis](image)

Table 4: Antioxidant activity of hexane extract of *C. alata* leaves

<table>
<thead>
<tr>
<th>Concentration of hexane <em>C. alata</em> extract (µg/ml)</th>
<th>% Inhibition of DPPH free radical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. alata</em> hexane extract</td>
</tr>
<tr>
<td>5</td>
<td>13.5±1.72</td>
</tr>
<tr>
<td>10</td>
<td>18.25±2.04</td>
</tr>
<tr>
<td>20</td>
<td>35.62±1.64</td>
</tr>
<tr>
<td>30</td>
<td>52.15±1.39</td>
</tr>
<tr>
<td>40</td>
<td>80.67±1.80</td>
</tr>
<tr>
<td>50</td>
<td>96.65±1.43</td>
</tr>
</tbody>
</table>

Antidermatophytic activity of leaves extract of *C. alata*

The results of antifungal activity of hexane extracts of *C. alata* leaves against the five different dermatophytic fungal strains are interpreted in Table 5 and Figure 6. The antifungal activity was assessed by zone formation around the well at different concentrations of hexane extracts.
extract. This test concluded that hexane extract show more activity against *Microsporum canis* with the zone formation range from 8.33±0.17 to 12.37±0.19 mm and minimum zone of inhibition was noted against *Microsporum audouinii*. In this study, zone of inhibition of extracts was increased as increasing the volume of the dosage. However, antifungal activity of extract is directly proportional to dosage of extract i.e. antifungal activity is increased as a dose dependent manner. The antifungal activity of hexane extract against microorganisms was arranged orderly by following: *M. canis* > *E. floccosum* > *T. rubrum* > *T. mentagrophytes* > *M. audouinii*.

![Figure 6: Anti-dermatophytic activity of hexane extract of C. alata leaves at different concentrations](image)

Table 5: Zone of inhibition and percentage of zone of inhibition of hexane extract of C. alata leaves against dermatophytes

<table>
<thead>
<tr>
<th>Dermatophytes</th>
<th>Zone of inhibition (mm in diameter)</th>
<th>% Zone of Inhibition</th>
<th>MFC and MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25µL</td>
<td>50µL</td>
<td>75µL</td>
</tr>
<tr>
<td><em>M. canis</em></td>
<td>08.33±0.17</td>
<td>09.17±0.17</td>
<td>12.37±0.19</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>06.17±0.17</td>
<td>06.73±0.37</td>
<td>08.23±0.15</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>06.06±0.07</td>
<td>06.20±0.12</td>
<td>08.23±0.17</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>06.20±0.10</td>
<td>08.23±0.15</td>
<td>11.33±0.18</td>
</tr>
<tr>
<td><em>M. audouinii</em></td>
<td>06.00±0.00</td>
<td>07.17±0.17</td>
<td>08.00±0.00</td>
</tr>
</tbody>
</table>

± Standard deviation; C – Control; control is considered as 100% of inhibition; Nf<100 – No fungicidal activity below 100 µl/mL.
**Percentage of zone of inhibition**
Terbinafine is used as positive control and its complete antifungal activity against pathogens is considered as 100% of inhibition. It was compared with hexane extract of *C. alata* leaves which shown 87.4% of inhibition against *M. canis* and minimum percentage zone of inhibition was observed against *M. audouinii* (62.4%). This result concluded that hexane extract have the ability to complete control against all the pathogens above 60% of inhibition (Table 6).

**MIC and MFC determination**
Minimum inhibitory concentration and minimum fungicidal concentration of hexane extract was revealed by mean diameter of zone of inhibition against dermatophytes. The results of antifungal activity of hexane crude extracts of *C. alata* leaves showed good activity on all the strains of *M. canis, T. mentagrophytes, T. rubrum, E. floccosum* and *M. audouinii* tested at different concentrations. However, hexane extract shown MIC and MFC values of 20.0 and 25.0 µl/ml were recorded against *T. mentagrophytes* (Table 6).

**CONCLUSION**
Naturally, the plant *C. alata* has enormous medicinal applications to curing harmful diseases. Our aim of this study is to identify the potential bioactive compounds involving in medicinal properties by performing general analytical techniques like GC-MS, HPLC and FTIR. The hexane extract showed high fungicidal effect against a dermatophytes *M. canis*. Preparation of fungicide from plants accepted as a natural source of alternative medicine to inhibit the growth human pathogenic fungi.

**REFERENCES**
3. Dimayuga RE and Garcia SK (1991) Antimicrobial screening of medicinal plants from Baja California sur, Mexico, J. Ethnopharmacol 31:181-192


