Study on The Pharmacognostic Characterization and Antimicrobial Activity of the Medicinal Plant \textit{Cassia obtusa} L.

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Abstract

The species, \textit{Cassia obtusa} L., consists of small herbs found in tropical and subtropical regions and have wide application in herbal formulations. Leaf, stem, and fruit are used to cure various ailments in human beings. In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines researches in bioactive substances might lead to the discovery of new compounds that could be used to formulate new and most potent antimicrobial drugs to overcome the problem of resistant to the currently available antibiotics.

The main objective of the present investigations is to analyze the fluorescence characters and evaluate the antimicrobial activity of crude extract of leaf, stem and fruit against selected gram positive and gram negative bacteria. The leaf, stem and fruit powder of the plants showed varying degree of antibacterial activity against all the tested bacteria.

Introduction

Medicinal plants played an important role in the discovery of novel and useful drugs used in modern medicine. Today we have a number of drugs useful and life saving and also drugs which can provide immediate therapeutic benefit (Dubey, \textit{et al.}, 2004). Over 2000 plant species are found to have medicinal value and are used for medicinal purpose in different forms. Many common plants seen in the Kitchen gardens or in the compound or in the forests are used by the tribal as medicines. (Rushulo Kemp, 2003).

Our country is bestowed with rich and diverse resources of plant wealth including an enormously large number of medicinal plants. They are extensively used as antitumor, immunomodulators, antidiabetics, Purgatives hepatoprotectives, anti-inflammatory, antioxidants and antidotes.

The objectives of the present investigation are to record the traditional uses of the medicinal plant, \textit{Cassia obtusa}.

Materials and Methods

The aim of present investigation was to evaluate the preliminary phytochemical characters such as determination of pharmacognostic and fluorescence characters, and in vitro antibacterial activity of the important medicinal plant \textit{Cassia obtusa} L.

Family : Caesalpinaceae
Tamil : Mulaipal Virai
Ayurveda : Chakshushya (Venakulatha)
Shidha : Kaatukollu (Idikollu)

Glandular hairy herbs, leaf lets 2 pairs, flower yellow, interminal or leaf opposed racemes, pod slightly oblique, hairy; seed trapezoid ovoid.

**Uses:** Seeds used in opthalmia and skin troubles, also used as a cathartic; leaves used in cough, constipation and wounds.

**Collection of Plant Material**

Fresh plants of *Cassia obtusa* L. were collected from Saduragiri hills, Virudhunagar district, Western Ghats, Tamil Nadu in the month of May 2007. The plants were identified by Gambles Flora of Madras.

**Pharmacognosy**

Pharmacognosy deals with identification of the source of the material forming dry, description of its morphology and anatomy investigation of its potency, purity and freedom from admixture.

**Determination of total ash value**

Two grams of the drug powder was accurately weighed in a silica crucible which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighted. The procedure was repeated to get a constant weight. The percentage of the total ash in calculated with reference to the air dried drug.

**Determination of acid insoluble ash**

The ash obtained in the determination of total ash was boiled for 5 minutes, with 25ml of HCL. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to a pre weighed Silica crucible and then ignited, cooled and weighed. The procedure was repeated to get constant weight.

**Method for fluorescence analysis**

Many phyto drugs when suitably illuminated emit light of different wave length or colour from that which falls on them. The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration. Fluorescence studies were done previously in *Boerhaavia diffusa* (Murugan and Murugesan, 2006).

**Antibacterial Assay**

**Collection of microorganisms**

Stock cultures of bacteria such as *Staphylococcus aureus, Streptococcus lactis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Vibrio cholerae* were obtained from Research laboratory, Department of Microbiology, Madura College, Madurai, Tamil Nadu.

**Preparation of media**

The growth media employed in the present study included Nutrient agar and Nutrient beef:

- **Beef extract** – 3.0 g
- **Peptone** - 5.0 g
- **Agar** - 15.0 g
Distilled water - 1000ml

Nutrient broth is composed of without Agar. The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min.

**Preparation of inoculum**

Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5ml of nutrient broth and incubated overnight at 37°C. These overnight cultures were used as inoculum.

**Antimicrobial activity**

Antimicrobial activity was demonstrated by modification of the method described by Barry and Thornsberry (1985).

**Results and Discussion**

**Pharmacognostic Characters**

The pharmacognostic characters of the medicinal plants *Cassia obtusa* are presented in Table 1. The pharmacognostic characters are helpful in evaluating the pharmacognostic value of the medicinal plants. The moisture content of *Cassia obtusa* leaf (76.4%) and fruit (72.8%). *Cassia obtusa* fruit was found to possess higher total ash content (6.01%).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Particulars of Parameters</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td>1.</td>
<td>Loss of weight on drying</td>
<td>76.4</td>
</tr>
<tr>
<td>2.</td>
<td>Total ash</td>
<td>5.43</td>
</tr>
<tr>
<td>3.</td>
<td>Acid soluble ash</td>
<td>2.16</td>
</tr>
<tr>
<td>4.</td>
<td>Water soluble ash</td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td><strong>Extracts</strong></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Hexane</td>
<td>1.08</td>
</tr>
<tr>
<td>6.</td>
<td>Benzene</td>
<td>1.13</td>
</tr>
<tr>
<td>7.</td>
<td>Methanol</td>
<td>1.24</td>
</tr>
</tbody>
</table>

**Table 1. Pharmacognostic characters of leaf and fruit of *Cassia obtusa*.**
Fluorescence analysis of *Cassia obtusa*

The powder and the crude extracts were examined under day light and Ultra violet light (365 nm). The results of the observation presented in Table 2.

**Table 2.** Fluorescence analysis of leaf and fruit powder and their extract in different solvents of *Cassia obtuse*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Leaf</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Visible / Day light</td>
<td>365 nm UV Light</td>
</tr>
<tr>
<td>1.</td>
<td>Drug powder as such</td>
<td>Pale yellow</td>
<td>Pale green</td>
</tr>
<tr>
<td>2.</td>
<td>Powder +1N NaOH (Aqueous)</td>
<td>Reddish orange</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + 1N NaOH (alcohol)</td>
<td>Reddish orange</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>4.</td>
<td>Powder + 1N HCl</td>
<td>Pale yellow</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>5.</td>
<td>Powder + 1% H₂SO₄</td>
<td>Blackish yellow</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>6.</td>
<td>Powder + 1% Nitric acid</td>
<td>Reddish orange</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>7.</td>
<td>Extracts Hexane</td>
<td>Pale yellow</td>
<td>Green</td>
</tr>
<tr>
<td>8.</td>
<td>Benzene</td>
<td>Greenish yellow</td>
<td>Green</td>
</tr>
<tr>
<td>9.</td>
<td>Methanol</td>
<td>Dark green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>10.</td>
<td>Water</td>
<td>Brown</td>
<td>Green</td>
</tr>
</tbody>
</table>

**Antimicrobial activity**

Screening for bioactive compounds from plants and product for the antimicrobial activity has shown that plants reported a potential source of new antimicrobials (Hernandez *et al.*, 1999; Srinivasan *et al.*, 2001). In the present investigation an attempt is made to analyse their antimicrobial response and the crude drugs used as the ethnomedicine.

The leaf and fruit extracts of *Cassia obtusa* was tested for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Vibrio cholerae*, and the results are presented.
Antibacterial activity of Cassia obtusa

Hexane extracts of leaf powder showed maximum inhibitory activity against Streptococcus lactis (3mm). Moderate inhibitory activity (2mm) was observed in hexane extracts of Cassia obtusa leaf powder against Staphylococcus aureus and Pseudomonas aeruginosa and V. cholerae. Very less inhibitory action (1mm) was observed in hexane extracts of leaf powder against E.coli.

Maximum inhibitory activity was observed against E. coli (4mm) and B. subtilis (3mm) in hexane extracts of fruit powder. Very less inhibition action (1mm) was observed in hexane extracts of fruit powders against S. aureus and V. cholerae.

Benzene extracts of leaf powder showed maximum inhibitory activity against S. lactis (3mm) and P. aeruginosa (3mm) Benzene extracts of leaf showed moderate activity against B. subtilis (2mm) and E. coli (2mm). No antibacterial activity was observed against S. aureus and V. cholerae.

Benzene extracts of fruit showed maximum inhibitory activity against E. coli (5mm) and V. cholerae (4mm) Moderate activity was observed against S. lactis (2mm) in benzene extracts of fruit powder. Very less antibacterial activity was observed against S. aureus (1mm).

Methanol extracts of leaf exhibited maximum inhibitory activity against P. aeruginosa (4mm), S. aureus (3mm) and V. cholerae (3mm). Mild antibacterial activity was observed against E. coli (2mm). Very less inhibitory activity was observed in methanol extracts of leaf powder against B. subtilis.

Maximum inhibitory action was observed in methanol extracts of fruit against V. cholerae (5mm). Moderate inhibitory action was observed against S. lactis (2mm) and E. coli (2mm). No inhibitory action was observed against B. subtilis.

Antibacterial activity was observed against S. aureus (2mm) in water extracts of leaf. Very less inhibitory action (1mm) was observed against B. subtils, P. aeruginosa, and V. cholerae.

Aqueous extracts of fruit powder showed the antibacterial activity against B. subtilis (3mm). Moderate activity was observed against E. coli (2mm). Very less inhibitory action was observed against S. aureus (1mm).

It was observed from the results that benzene and methanol extracts of fruit exhibited maximum inhibitory action against gram negative bacteria E. coli (5mm) and V. cholerae (5mm) when compared with other extracts.

References