Pharmacognostic, Phytochemical and Pharmacological Studies of *Cassia roxburghii*

Pallavi Kamarapu*
Department of Pharmacognosy, Vaagdevi College of Pharmacy, Kakatiya University, Warangal, Telangana, 506009, India

**Abstract**

In the present study, the leaf of *Cassia roxburghii* and its powder were subjected to pharmacognostic evaluation in terms of macroscopic and microscopic evaluation. The powdered drug was subjected to extraction with various solvents such as petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract by successive maceration. The methanolic and aqueous leaf extracts exhibited reducing power compared with ascorbic acid at similar concentrations. Based on the biochemical estimation of serum marker enzymes and histological study, all the extracts showed hepatoprotective activity and the maximum activity was seen in methanol extract at 200 mg/kg. The order of activity Me200＞aq200＞Me400＞Aq400.

**Keywords:** Pharmacognostic evaluation; *Cassia roxburghii*; Enzymes

**Introduction**

The liver is the major organ in the body, contributing about 1/50 of the total weight of the body. It lies in the upper part of the abdominal cavity. More than 500 vital functions have been identified with the liver. The liver is important because a person’s nutritional level is not only determined by what he or she eats, but by what the liver processes. It is difficult to detect symptoms of liver metabolic imbalances. Some of the common disorders of the liver include cirrhosis, viral hepatitis, alcoholic liver disease, hemochromatosis, liver cancer, jaundice and drug induced liver damage. Beyond the treatment of liver disorders, everyday care of the liver lays a cornerstone for total body health. Platonic and others, who look beneath the symptoms of an illness to its underlying cause, often discover that the liver had a role to play. People can suffer for a long time from a liver ailment without knowing of it. The incredible complexity of liver chemistry and its fundamental role in human physiology is so daunting to researchers that the thought that simple plant remedies might have something to offer is astonishing and incredible [1-5].

*Cassia* species have important role in phytochemical and pharmacological research due to their excellent medicinal values. Different classes of natural products, possessing potent physiological and pharmacological activities have been isolated from *cassia* species and they include anthracen derivatives, flavonoids and poly saccharides. Some of these compounds have been shown to possess Considerable antimicrobial activity. *Cassia* species are well known in folk medicine for their laxative and purgative uses. They are also used for treating skin diseases such as ring worm, scabies, eczema and wounds [6].

**Materials and Methods**

**Collection of plant material**

*Cassia roxburghii* is a uncommon in cultivation. The plant material collected from madikonda local areas of Warangal, India. Its parts were botanically authenticated by Taxonomist, Department of Botany, Kakatiya University, Warangal, India. A voucher specimen (CV-028) was maintained in the Department of Pharmacognosy and Phytochemistry, Vaagdevi College of pharmacy, India

**Macroscopic, microscopic and physical evaluation of plant material**

Macroscopic, Microscopic and physical evaluation of plant material was done by performing organoleptic, T.S, ash and extractive values.

**Preparation of plant material**

*Cassia roxburghii* leaves were washed under tap water and were efficiently dried under shade for about one week and protected from deterioration. The shade dried leaves were grinded made into powder with the help of blender.

**Extraction**

**Maceration**

The leaf material was weighed (250 g) and extracted by maceration using the solvents petroleum ether, chloroform, ethylacetate, methanol and aqueous at room temperature in a glass container for 3 days. The material was stirred from time to time to ensure proper extraction. After 3 days, the contents of the container were filtered through muslin cloth and the filtrate was concentrated under reduced pressure below 500°C, until a soft mass obtained and then preserved in a desiccator. Finally phytochemical screening was done by performing the different identification tests [7-9].

**Reducing power method**

Different concentrations of the extracts from (100 µg/ml-1000 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml pH 6.8) and potassium ferricyanide (2.5 ml 1%). Then incubated at 500°C for 20 min. To this, *trichloroacetic acid* was added and centrifuged at 3000 rpm for 10 min.

Then the upper layer was added with distilled water (2.5 ml), FeCl3 (0.5 ml 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

*Corresponding author:* Pallavi Kamarapu, Department of Pharmacognosy, Vaagdevi College of Pharmacy, Kakatiya University, Warangal, Telangana, 506009, India, E-mail: pallaviudheer2008@gmail.com

**Received:** April 10, 2015; **Accepted:** April 23, 2015; **Published:** April 30, 2015

**Citation:** Kamarapu P (2015) Pharmacognostic, Phytochemical and Pharmacological Studies of *Cassia roxburghii*. J Bioengineer & Biomedical Sci 5: 152. doi:10.4172/2155-9538.1000152

**Copyright:** © 2015 Kamarapu P. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
H$_2$O$_2$ scavenging assay method

H$_2$O$_2$ (43 mM) was prepared in phosphate buffer saline (pH 7.4). Positive control (Ascorbic acid) and extract solutions were prepared at concentrations of 50-250 µg/ml. Aliquots (different concentrations) of standard and extracts solutions (3.4 ml) were added to 0.6 ml of H$_2$O$_2$ solution. The reaction mixture was incubated at room temperature for 10 min and the absorbance was determined at 230 nm [10-13].

The % of scavenging was calculated as follows: % H$_2$O$_2$ scavenging = 100 X (absorbance of control – absorbance of sample)/absorbance of Control.

Acute toxicity testing

Acute toxicity and gross behavioral studies was carried out in mice after administration of various extracts of leaf of Cassia roxburghii. Albino mice weighing 20-25 gm were selected, weighed and marked. The mice were kept on overnight fasting before going to the test. The mice were divided into five groups with each group containing 6 mice. The test extracts were given orally in the form of suspension in arachis oil. The five groups of mice received the doses of extracts at 200, 400, 800 and 2000 mg/kg. Then the mice were continuously and carefully observed for 2 hrs followed by occasionally for further 4 hrs. The behavior and mortality of mice was observed up to 24 hrs. A one week washout period was allowed after studying each extract. Observation of behavioral changes will guide to go for further screening for the proposed activities [14].

Hepatoprotective Activity

In the present study, the animals were pretreated with test extracts and a standard drug silymarin (100 mg/kg) before inducing liver damage with CCl$_4$. The duration of the study was seven days. After acclimatization the rats were divided into thirteen groups (I-XIII). Each group consisting of six animals. All animals were kept on same diet for 7 days. The division of animals for Hepatoprotective activity of leaf extract of Cassia roxburghii is as follows.

Group I served as normal and received 1 ml/kg of arachis oil p.o. for seven days. Group II served as toxic control and was given 5 ml/kg of 50% v/v CCl$_4$ in olive oil i.p. on the seventh day. Group III (standard) animals were administered with 100 mg/kg of silymarin p.o. for seven days, followed by CCl$_4$ administration i.p. on the seventh day. Group IV–VII were treated in a similar way to that of group III (standard) using methanol extract and aqueous extract of leaf of Cassia roxburghii at doses of 200 mg/kg and 400 mg/kg in place of standard respectively, followed by CCl$_4$ administration i.p. on seventh day. All the rats were anaesthetized with thiopentone sodium (60 mg/kg i.p.) 36 h after administration of CCl$_4$. Then blood was collected from common carotid artery by carefully opening the neck region of the rat. After blood collection, the blood samples were allowed to coagulate on hot plate to fix liver sections onto the slides. The slides were then placed for 5 minutes each in xylene to remove wax, then in absolute alcohol to remove xylene from the liver sections. Hydration of liver sections was attained by keeping them in descending series of alcohol and water mixtures (90%, 70%, 50%, 30% alcohol and in pure water) for three minutes each. Hydrated sections were stained with haematoxylin stain for one minute and washed in running tap water to remove excess stain. Liver sections were dehydrated again by keeping in ascending of alcohol mixtures (30%, 50%, 70%, 90% alcohol) for one minute. After that, the sections were kept for 5 minutes each in absolute alcohol and then in xylene. Finally, the stained liver sections were mounted in DPX (Desterenedibutyls phthalate xylene) and viewed under optical microscope for histological examination [21-24].

Results and Discussion

In this evaluation microscopy and macroscopy of leaf of Cassia roxburghii were studied. The observations of the investigations were, the leaf powder of the plant was studied for their organoleptic characters like colour, odour and taste. The results of this study were Color – Greenish, Taste – mucilaginous, Odour - Characteristic

The leaf powder of the plant has shown the presence of following plant tissue systems under microscopic evaluation:

- Calcium oxalate crystals : Prismatic type
- Starch grains : Simple, compound
- Vascular tissue : Xylem and phloem
- Trichomes : Unicellular covering trichomes

Quantitative microscopy of leaf/leaf powder Cassia roxburghii: The powder analysis of leaf powder of the plant was evaluated and the results obtained were shown below. The width range of phloem fibers in powdered leaf of Cassia roxburghii was found to be 15.46 µ. The diameter of starch grains in powdered leaf of Cassia roxburghii was found to be 2.45 µ (Simple and compound). The length of the calcium oxalate crystals in powdered leaf of Cassia roxburghii was found to be 3.2 µ. Vascular bundle: Xylem and Phloem (Figures 1-3).

Transverse section of leaf

- Rubiaceous or Paracytic stomata.
- Spongy tissue of parenchymatous cells.
- Cluster crystals of calcium oxalate (prismatic type) in palisade and crystal sheath in mid rib region.
- Palisade-a single layer below upper and lower epidermis.
- Epidermal trichomes-Unicellular covering trichomes.
Epidermis-beaded walled epidermis (Figure 4 and Tables 1-3).

Acute toxicity studies were performed for all the five extracts at doses of 200, 400, 800 and 2000 mg/kg body weight in mice. The behavioral changes were observed for 24 hours. The observation of the behavioral changes the animals did not show any toxic effects up to the dose of 2000 mg/kg.

Antioxidant activity

Reducing power method (Tables 4-6 and Figure 5): The reducing power of methanolic, aqueous extracts were 1.01, 0.55 at 1 mg/ml respectively however at 1 mg/ml ascorbic acid showed excellent reducing power of 1.01.
Ascorbic acid concentrations (µg/ml) Absorbance at 230 nm % Inhibition

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.146</td>
<td>16.04</td>
</tr>
<tr>
<td>100</td>
<td>0.26</td>
<td>28.5</td>
</tr>
<tr>
<td>150</td>
<td>0.422</td>
<td>46.3</td>
</tr>
<tr>
<td>200</td>
<td>0.818</td>
<td>89.8</td>
</tr>
<tr>
<td>250</td>
<td>0.91</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 7: Table showing ascorbic acid % inhibition.

Leaf aqueous extract concentrations (µg/ml) Absorbance at 230 nm % Inhibition

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.209</td>
<td>22.4</td>
</tr>
<tr>
<td>100</td>
<td>0.399</td>
<td>42.8</td>
</tr>
<tr>
<td>150</td>
<td>0.483</td>
<td>51.8</td>
</tr>
<tr>
<td>200</td>
<td>0.517</td>
<td>55.5</td>
</tr>
<tr>
<td>250</td>
<td>0.931</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 8: Table showing leaf aqueous extract % inhibition.

Leaf methanolic extract concentrations (µg/ml) Absorbance at 230 nm % Inhibition

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.728</td>
<td>71.02</td>
</tr>
<tr>
<td>100</td>
<td>0.736</td>
<td>71.80</td>
</tr>
<tr>
<td>150</td>
<td>0.804</td>
<td>78.43</td>
</tr>
<tr>
<td>200</td>
<td>0.904</td>
<td>88.19</td>
</tr>
<tr>
<td>250</td>
<td>1.025</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 9: Table showing leaf methanolic extract % inhibition.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>IC50 Values(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>136.633</td>
</tr>
<tr>
<td>2</td>
<td>Methanol extract of Cassia roxburghii</td>
<td>110.375</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous extract of Cassia roxburghii</td>
<td>136.599</td>
</tr>
</tbody>
</table>

Table 10: Table showing IC50 values.

0.91, respectively which are significantly higher than that of methanol and aqueous extracts. In the present study methanolic extract showed higher reducing power than Aqueous extract.

H2O2 scavenging assay method (Tables 7-10 and Figure 6)

The methanolic and aqueous whole plant extracts exhibited higher H2O2 scavenging activity than ascorbic acid at similar concentrations. The IC50 values of the methanolic and aqueous extracts of leaves and ascorbic acid were 110.375, 136.599 and 136.633 µg/ml, respectively (Table 11 and Figures 7-10).

Histological study

Shown in Table 12 and Figures 11-14.

Conclusion

Whole plant and seeds are used to treat skin diseases like ringworm, antimicrobial, hepatoprotectivity. The methanolic and aqueous leaf extracts both exhibited Antioxidant activity compared with ascorbic acid. Methanolic extract found to be more potent Antioxidant activity compared with aqueous leaf extract. All the leaf extract of Cassia roxburghii showed hepatoprotective activity and methanolic extract (200 mg/kg) was found to be more potent hepatoprotective compare with the other extracts. The histological studies also indicated that methanol extract (200 mg/kg) showed significant activity when compared with other extracts. Therefore, it can be concluded that Cassia roxburghii may have “potential hepatoprotective activity thus further mechanism based studies are needed for its hepatoprotective activity”.

S.No | Particulars | IC50 Values(µg/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>136.633</td>
</tr>
<tr>
<td>2</td>
<td>Methanol extract of Cassia roxburghii</td>
<td>110.375</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous extract of Cassia roxburghii</td>
<td>136.599</td>
</tr>
</tbody>
</table>

Table 10: Table showing IC50 values.
Table 11: Effect of different leaf extracts of Cassia roxburghii on serum biochemical parameters in CCl4 induced liver toxicity.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Group</th>
<th>Score</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>Showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein.</td>
</tr>
<tr>
<td>2</td>
<td>CCl4</td>
<td>4</td>
<td>Showed necrosis, steatosis, fatty vacuolization and pseudolobule formation were seen.</td>
</tr>
<tr>
<td>3</td>
<td>Reference standard</td>
<td>0</td>
<td>Showed significant restoration of liver architecture comparable with normal rats.</td>
</tr>
<tr>
<td>4</td>
<td>Methanolic (200 mg/kg)</td>
<td>1</td>
<td>Showed almost normal liver architecture with mild degree of fatty changes compared with normal and standard.</td>
</tr>
<tr>
<td>5</td>
<td>Methanolic (400 mg/kg)</td>
<td>3</td>
<td>Moderated restoration of liver architecture with mild degree of necrosis.</td>
</tr>
<tr>
<td>6</td>
<td>Aqueous (200 mg/kg)</td>
<td>2</td>
<td>Showed mild fatty change and mild sinusoidal congestion.</td>
</tr>
<tr>
<td>7</td>
<td>Aqueous (400 mg/kg)</td>
<td>3</td>
<td>Showed mild degree of necrosis with areas of inflammation.</td>
</tr>
</tbody>
</table>

Table 12: Grading of liver damage based on histological study.
References