ANTHOCYANIN CONTENT OF ETHANOLIC EXTRACTS OF THREE SPECIES OF CASSIA (LEGUMINOSAE) FROM SOUTHERN INDIA


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Abstract

Cassia is a vast genus with many different species, which are distributed throughout the southern part of India. Cassia has great potential as a source of anthocyanins. C. obovata, C. sylvestris, and C. acutifolia are three species of the genus Cassia and are distributed in the south of India. The present study was aimed at determining the anthocyanin content of these species. The results show that C. obovata, C. sylvestris, and C. acutifolia are potential sources of anthocyanins. The anthocyanin content of C. obovata was found to be the highest among the three species, while C. acutifolia had the lowest content.

Key words: Cassia, Anthocyanins, Ethanol extracts, Southern India.

Introduction

Cassia is a genus of flowering plants in the family Leguminosae, which is distributed throughout the southern part of India. Cassia is a rich source of anthocyanins, which are important for their antioxidant and anti-inflammatory properties. The present study was aimed at determining the anthocyanin content of three species of Cassia, namely C. obovata, C. sylvestris, and C. acutifolia, which are distributed in the south of India. The results show that C. obovata, C. sylvestris, and C. acutifolia are potential sources of anthocyanins. The anthocyanin content of C. obovata was found to be the highest among the three species, while C. acutifolia had the lowest content.
It has been used in Indian traditional system of medicine since a long time in diseases due to vitiated blood, skin diseases and ulcers. The herb is used in folklore as galactogogue, cholagogue, abortifacient and febrifuge. In some parts of Bihar, the plant is used for hazy vision, night blindness, diarrhoea, dysentery and post-natal complaints. The poultice of pounded fresh material is reported to be used for sprains, burns, eczema, carbuncle, erysipelas and acute conjunctivitis. It is applied externally on acne and pimples. Previous phytochemical studies have reported the isolation of flavonols, triterpenoids, steroids and tannins; β-sitosterol, stigmasterol, campesterol, lupeol being few of its important constituents.[8] The herb has been reported to have antipyretic[8], hepatoprotective[9], antiulcer[10], antibacterial[11], hematinic[12], diuretic[13] activities. Taking into consideration the folklore uses and the active constituents present, the present study aims at pharmacological evaluation of ethanolic and aqueous extracts of *Alternanthera sessilis* (L) for anti-inflammatory activity.

Materials and methods

**Plant material**: Fresh aerial parts of *Alternanthera sessilis* were collected in July 2010 and the plant was authenticated by Dr. Shiddamallayya N from Regional Research Institute (Ay.) (Central Council of Research in Ayurveda and Sidha, Dept of AYUSH, Ministry of Health and Family Welfare, Govt. Of India, New Delhi), Government Central Pharmacy, Annexe, Ashoka Pillar, Jayanagar, Bangalore-560 011.

**Preparation of extract**: Fresh aerial parts (leaves, flowers, stem) of *Alternanthera sessilis* Linn. were procured, shade dried, coarsely powdered and was successively Soxhlet extracted with petroleum ether, chloroform and 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic extracts (7.7%) and aqueous extracts (9.9%).

**Animals**: albino Wistar rats were purchased from M/s Venkateshawara Traders, Bangalore-560 010 and were maintained under standard animal house conditions in animal house of KLE University’s College of Pharmacy, Bangalore. Experimental protocol was approved by Institutional animal ethics committee (IAEC) of KLE University’s College of Pharmacy, Bangalore.

**Acute toxicity study [14]**: Acute toxicity studies for ethanolic and aqueous extracts were conducted as per OECD guidelines 425 to determine the safe dose using female albino Wistar rats weighing 150-200g. No sign and symptoms of toxicity were observed during the observations which was done continuously for the first 4h and then observed up to 24h for mortality. The extracts were safe up to a dose of 2000mg/kg b.w. The biological evaluation was carried out at doses of 200 and 400mg/kg b.w.

**Preliminary phytochemical screening [15]**:

The ethanolic and aqueous extracts of *Alternanthera sessilis* Linn. were subjected to preliminary phytochemical screening.

**Anti-inflammatory activity**: Anti-inflammatory activity was determined by Carrageenan induced rat paw edema model.

**Carrageenan induced inflammation[16]**:

Animals in the range of 150-200g were divided into VI groups of 6 animals each. Group I served as control, animals of Groups II to VI received Indomethacin at the dose of 10mg/kg b.w., aqueous and ethanolic extracts (200mg/kg b.w. and 400mg/kg b.w) of *Alternanthera sessilis* respectively.

After 1h of drug administration, 0.1ml carrageenan (1% suspension) was injected into the sub-plantar region of the right hind paw of each rat. The paw volumes were measured at 0, 1, 2, 3, 4, 5, 6h intervals with the help of plethysmometer. The percentage decrease in paw volume was determined using the formula:

\[(\text{Control reading}-\text{Test reading})/\text{Control reading} \times 100\]
Statistical analysis: The interpretation of the results was done after subjecting the data obtained from various studies to statistical analysis which included one way ANOVA followed by post test Tukey.

Results

Carrageenan induced paw edema:
The anti-inflammatory activity of ethanolic and aqueous extracts and Indomethacin started from 1\textsuperscript{st} h and continued up to 6\textsuperscript{th} h. The percentage inhibition of edema produced during 1\textsuperscript{st} h by ethanolic and aqueous extracts was comparable to that of standard. Maximum inhibition of edema was produced at 3\textsuperscript{rd} h. Maximum inhibition of edema was produced by ASE400 (64.29±7.14) followed by Indomethacin and ASA400 (57.14±4.24 and 57.14±8.24). At 4\textsuperscript{th} h, the percentage inhibition of edema produced by ASE400 was comparable to that of Indomethacin. In the 5\textsuperscript{th} h, Indomethacin produced maximum inhibition of edema (55.56±0) followed by ASE400 and ASA400 (44.44±8.12 and 44.44±8.12). (Table 1)

Table 1: Anti-inflammatory activity of aqueous and ethanolic extracts of *Alternanthera sessilis* on carrageenan induced paw edema in albino Wistar rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (kg(^{-1}))</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>60±0</td>
<td>54.55±5.09</td>
<td>57.14±4.24</td>
<td>54.55±5.09</td>
<td>55.56±0</td>
<td>37.5±4.27</td>
</tr>
<tr>
<td>ASA</td>
<td>200</td>
<td>30±10</td>
<td>27.27±0</td>
<td>21.43±7.14</td>
<td>27.27±0</td>
<td>33.33±6.83</td>
<td>12.5±5.25</td>
</tr>
<tr>
<td>ASA</td>
<td>400</td>
<td>50±10</td>
<td>36.36±9.09</td>
<td>57.14±8.24</td>
<td>45.45±10.5</td>
<td>44.44±8.12</td>
<td>50±0</td>
</tr>
<tr>
<td>ASE</td>
<td>400</td>
<td>50±10</td>
<td>45.45±10.5</td>
<td>64.29±7.14</td>
<td>54.55±9.09</td>
<td>44.44±8.12</td>
<td>50±0</td>
</tr>
</tbody>
</table>

n=6, values are mean±SEM, where ASA and ASE indicates *Alternanthera sessilis* aqueous and ethanolic extracts respectively.

Discussion

Inflammation is the reaction of a tissue and its microcirculation to a pathogenic insult. By this mechanism the host localizes and eliminates metabolically altered cells, foreign particles, microorganisms or antigens.[17] Mediators of inflammation include histamine, bradykinin, prostaglandins, Leukotrienes, platelet activating factor, interleukin-1, Thromboxane A\(_2\) and prostacyclin.[18]
Carrageenan induced paw edema is an in-vivo model of inflammation, it has been frequently used to assess the anti-inflammatory activity of steroidal and non-steroidal drugs involving inhibition of several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins.[19] The results of the present study reveals the anti-inflammatory activity of Alternanthera sessilis on acute phase of inflammation induced by carrageenan.[20] The acute inflammatory responses induced by carrageenan administration involves three phases of chemical mediator release in an orderly sequence. For the first 1.5h an intial phase takes place with the release of histamine and serotonin and for the subsequent 1.5-2.5h a second phase is mediated by bradykinin. The third and final phase occurs between 2.5 and 6h and is presumably mediated by prostaglandins (PGs).[21] Besides, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism. Therefore, it is suggested that the mechanism of action of Alternanthera sessilis may be related to the prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of Indomethacin in the inhibition of the inflammatory process induced by carrageenan.[20]

**Conclusion**

Alternanthera sessilis produced good anti-inflammatory activity. It can be considered to be used in therapeutics for treating inflammation as it has shown promising anti-inflammatory activity.

**Acknowledgement**

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**References**