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Phytochemical Study, Antimicrobial and Anticancerous Activity of Cassia tora Linn
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The knowledge of using herbal flora in the ethnic communities is descended from one generation to another. Ethnomedical plants are the area of research to find out alternative medicine of drugs to avoid emergence of multidrug resistance. Now a day, it is becoming a threat to people in most of clinical cases. A traditional Indian medical practice using plants is known as Ayurveda. Lots of plants used to cure different diseases using their natural healing and restoring properties are mentioned in the Ayurveda. People of different tribal communities inhabiting in India follow different cultures, rituals and knowledge of using traditional medicinal plants. Vast ranges of organic compounds present in the plants have been classified as primary and secondary metabolites. Primary metabolites of plants played essential roles in photosynthesis, respiration, growth and development. Phytosterols, acyl lipids, nucleotides, amino acids and organic acids are the major primary metabolites. Some other phytochemicals accumulated in the plant species are known as secondary metabolites. Secondary metabolites are playing a major role in plant defence system as they provide protection against herbivores, microbial infection, pollinators and seed dispersing animals. Some secondary metabolites are used as dyes; fibres, glues, oils, waxes, drugs, perfumes as well as they are considered as sources of new natural drugs, antibiotics, insecticides and herbicides [1]. Cassia tora Linn. is an ayurvedic plant, belongs to the family Leguminaceae and sub-family Caesalpiniaceae [2]. So, the present research has been selected to study the pharmacological activities of the plant such as antimicrobial and anticancer activities.

Phytochemical analyses
The present study preliminary focussed on some preliminary phytochemical tests to identify the presence of secondary metabolites in the leaf extract and seed extract of C. tora plant. Positive result for alkaloid, phenol, saponin, carbohydrate, glycoside and protein test (Tables 1 and 2) indicated the presence of those secondary metabolites [3-7]. In this study, it was also observed that ethyl acetate fraction of leaf extract showed strong positive result for phenol in compare to hexane fraction leaf extract and aqueous fraction of leaf extract. In this study, it was observed that leaf extract in ethyl acetate solvent showed strong positive result for phenol test in compare to hexane fraction and aqueous fraction of leaf extract. On the other hand, seed extract in ethyl acetate, hexane and aqueous fraction showed strong positive result for phenol test in compare to ethyl acetate fraction and aqueous fraction of seed extract.

Table 1: Leaf extract of C. tora showed different types of secondary metabolites.

<table>
<thead>
<tr>
<th>Cassia tora Leaf extract</th>
<th>Alkaloid</th>
<th>Phenol</th>
<th>Saponin</th>
<th>Carbo-hydrate</th>
<th>Glyco-sides</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexane</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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The crude extracts of leaf and seed of the plants were prepared separately using aqueous as well as organic solvent mainly ethyl acetate and hexane to study the pharmacological activities of the plant such as antimicrobial and anti-cancerous activities.
`+` indicates presence of particular phytochemical.

`++` indicates strong presence of particular phytochemical.

**Table 2:** Seed extract of *C. tora* showed different types of secondary metabolites.

<table>
<thead>
<tr>
<th>Cassia tora seed extract</th>
<th>Alkaloid</th>
<th>Phenol</th>
<th>Saponin</th>
<th>Carbo-hydrate</th>
<th>Glyco-sides</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexane</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

acetate solvent showed strong positive result for carbohydrate test in compare to hexane and aqueous fraction of seed extract.

Secondary metabolites such as alkaloid, phenol, saponin, carbohydrate, glycoside and protein present in leaf and seed extract of ethyl acetate, hexane and aqueous solvent.

**Phenolic content**

The phenolic content in leaf and seed extract of *C. tora* plant was measured \(^8\) by extrapolation of concentration of phenolic per ml extract using data of optical density of each extract from standard curve (Figure 1) of commercial phenol. It was evaluated that phenolic content maximum in both ethyl acetate leaf extract and seed i.e. 1300 µg and 900 µg phenolic per ml respectively, whereas 650 µg and 700 µg phenolic per ml in aqueous leaf extract and 50 µg and 50 µg phenolic per ml respectively. From the standard curve, it was evaluated that the total phenolic concentration of ethyl acetate leaf and seed extract of *C. tora* plant were 1300 µg/ml (optical density 0.57) and 900 µg/ml (O.D. 0.39) respectively; phenolic concentration of aqueous leaf and seed extract of *C. tora* plant were 650 µg/ml and 700 µg/ml. while phenolic concentration of hexane-leaf and hexane-seed extract of *C. tora* plant was 50 µg/ml.

**Antimicrobial activity**

Most of the antimicrobial activities were found with the organic solvents \(^4,5,9\). The leaf and seed extracts showed significant antimicrobial activity by effective zone of inhibition. From this study it was evidenced that *C. tora* leaf and seed extract have intense antimicrobial activity against a few pathogenic bacteria like *Klebsiella oxytoca*, *Salmonella typhi* and *Pseudomonas aeruginosa* as well as antifungal activity against *Aspergillus niger* and *Curvularia lunata*. Ethyl acetate leaf extract of *C. tora* plant showed maximum zone of inhibition against *Salmonella typhi* and *Pseudomonas aeruginosa*. Ethyl acetate leaf extract of *C. tora* showed maximum zone of inhibition against *Curvularia lunata*.

**Anti-cancer activity**

Effect of ethyl acetate fraction of leaf extract and hexane fraction of leaf extract on breast cancer cell line MCF7 was observed by MTT assay \(^10\). During this study, investigation of anticancer activity of *C. tora* leaf and seed extract was done which revealed the effect of hexane fraction and ethyl acetate fraction of leaf respectively on MCF7 breast cancer cell line using MTT assay. Half dilution and one-fourth dilution of hexane leaf extract showed 42% and 55% cell viability after treatment of cancer cell line i.e. less percentage of cell viability. The cell viability of cancer cell line treated with ethyl acetate fraction of half dilution and one-fourth dilution of leaf extract is 48% and 77% respectively i.e. less effective than hexane fraction of leaf extract of same dilutions as shown in (Figure 2). It indicated that the hexane fraction of leaf extract showed more effective anticancer activity than ethyl acetate fraction.
Figure 2: Percentage of cell viability of cancerous cells treated with ethyl acetate and hexane fraction of C. tora leaf extract separately.

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