ABSTRACT

*Cassia tora* Linn. (Leguminosae) is well known plant widely distributed in India and other tropical countries. It is an annual shrub and grows in wild wasteland. Different parts of the herbal drug are used for their medicinal values. It is well known traditional medicine as laxative and is recommended for treatment of leprosy, psoriasis, ophthalmic, skin diseases and liver disorders. Several phytoconstituents such as anthraquinone glycosides, naphthopyrone glycosides, phenolic compounds, flavonoids etc. have been reported in this plant. The present study was designed to establish quality standards of *C. tora*. Different physiochemical parameters like extractive values, ash values, were determined. Preliminary phytochemical screening was performed to detect different phytoconstituents. Preliminary phytochemical screening of the extracts in different solvent revealed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids and proteins. Pesticides residues and aflatoxins were determined but not found in the tested samples. Heavy metals were also determined and found within acceptable limits.
Total phenolics and flavonoids content were quantified. Establishment of quality standards will assist in quality, purity and sample identification of *C. tora* leaves. The data generated will be useful in development of Pharmacopoeial monographs.

**KEYWORDS:** *Cassia tora*, Phytochemical screening, Heavy metal , Pesticides, Aflatoxins.

**1. INTRODUCTION**

World Health Organization (WHO) encourages, recommends and promotes traditional or herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (Chaudhury, 1999; Raina, 2003). Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of an herbal drug (Patra et al., 2002). The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health and to ensure reproducible quality of herbal medicine (Straus, 2002).

*Cassia tora* Linn. (Leguminosae) is an annual herb, 30-39 cm high growing in India as wasteland rainy season weed. *C. tora* has known to possess various biological and pharmacological activities including antihepatotoxic (Wong *et al.*, 1989), antiallergic (Zhang and Yu, 2003), antimitagenic (Choi *et al.*, 1997), antioxidant (Zhenbao *et al.*, 2007), antibacterial (Sharma *et al.*, 2010) and antifungal (Mukherjee *et al.*, 1996). It is a rich source of anthraquinone glycosides and flavonoids (Anonymous, 1992; Vadyaratnam, 1997). This study has not been reported for the leaves of this plant. Such studies are essential for the establishments of its identity, quality and purity. Current study was therefore designed to provide requisite data that will be used in industries and councils for the development of pharmacopoeial monographs.
2. MATERIALS AND METHODS

2.1. Collection, authentication and preparation of sample

The leaves of *Cassia tora* L were collected from herbal garden, Jamia Hamdard, New Delhi and Identified and authenticated by taxonomist. A voucher specimen No.CT/FP-JH-115 was deposited in department of pharmacognosy and phytochemistry, faculty of Pharmacy, Jamia Hamdard, New Delhi for future reference. The leaves were thoroughly washed with water to get rid of the adherent impurities. The clean leaves were used for the preparation of extracts. The leaves were dried below 60ºC in hot air oven (Yorco-YSI-430) and powdered in mixer grinder (Philips-HL7710-600W). The obtained powder was passed through sieve no 14. The dried powdered (200gm) was subjected to extraction with different solvents (petroleum ether, n-butanol, chloroform, acetone and methanol).

2.2. Determination of extractive value

It is the amount of soluble constituents extracted with different solvents from a given amount of medicinal plant material. (Harborne, 1992; Mukherjee, 2002).

2.2.1. Maceration

The powder (20gm) was macerated with solvent (Petroleum ether, chloroform, methanol and water) of volume 100 mL in a closed flask for 48 hrs, shaking frequently during six hrs and allowing standing for 48 hrs. It is filtered rapidly, taking precaution against loss of solvent. The extracts obtained were filtered and evaporated to dryness by Rota evaporator (Buchi, Rotavapor-R-210 Switzerland) to constant weight and percentage yield was calculated. The extracts were stored at low temperature for further studies.

2.2.2. Hot Extraction

The powdered material of the drug (20 gm) was packed in a Soxhlet apparatus separately for each solvent like petroleum ether, chloroform and methanol. The aqueous extract was
prepared by reflux method. It is filtered rapidly, taking precaution against loss of solvent. The extracts obtained were filtered and evaporated to dryness by Rota evaporator (Buchi, Rotavapor-R-210 Switzerland) to constant weight and percentage yield was calculated. The extracts were stored at low temperature for further studies.

2.2.3. Successive Extraction
The dried powdered material (20 gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, acetone and methanol. The extracts were evaporated to dryness and their constant extractive values were recorded.

2.3. Determination of ash values
This constraint can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO₂ leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly materials. The water-soluble ash is used to estimate the amount of inorganic elements.

2.3.1. Determination of total ash values
The ground drug was incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content. Ignition of medicinal plant material yields total ash constituting both physiological (from the plant tissue) and non-physiological (extraneous matter adhering to the plant) ash.

2.3.2. Determination of acid insoluble ash values
Weighed ash was boiled with 25 ml dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight. Acid insoluble ash represents sand and siliceous earth.
2.3.3. Determination of water-soluble ash values
Weighed ash was dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained.

2.4. Phytochemical screening
The different extracts (Petroleum ether, chloroform, acetone, methanolic, aqueous) were subjected to preliminary phytochemical screening for the detection of secondary metabolites. The screening was performed for Alkaloids, anthraquinone glycosides, carbohydrates, phenolic compounds, flavonoids, protein, saponins, resins and lipids or fats. (Mukherjee, 2002).

2.5. Determination of total phenolic content
Total phenols were determined by Folin Ciocalteu reagent (Pourmorad et al., 2006). A dilute extract of plant extract (0.5 ml of 10mg/ml) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na2CO3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm (Schimadzu UV-Vis 1601). The standard curve was prepared using 10, 20, 40, 60, 80, 100 μg/ml solutions of gallic acid in methanol.

2.6. Determination of total flavonoid content
Aluminum chloride colorimetric method was used for flavonoids determination (Pourmorad et al., 2006). Plant extract (0.5 ml of 10mg/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm (Schimadzu UV-Vis 1601). The calibration curve was prepared by preparing rutin solutions at concentrations 10 to 100μg/ml in methanol.

2.7. Determination of heavy metal residues
Analysis of toxic heavy metals (arsenic, cadmium, lead and mercury) was performed by Atomic Absorption Spectrophotometer (AAS) according to the American Organization of Analytical Chemists (AOAC) (Anonymous, 2002; Anonymous, 2003).
2.8. Determination of pesticide residues

Determination of pesticides (organophosphates organochlorines and pyrethroids) residue was carried out by GC-MS as per the guideline of American Organization of Analytical Chemists (AOAC) (Anonymous, 2002; Anonymous, 2003).

2.9. Determination of Aflatoxins

Aflatoxins were analyses by HPLC method as mention in American Organization of Analytical Chemists (AOAC) guidelines (Anonymous, 2002; Anonymous, 2003).

3. RESULTS AND DISCUSSION

3.1. Physicochemical standardization

Physicochemical parameters include extractive values, total ash value, acid insoluble ash value and water soluble ash value were determined to check the purity of the drug. The results of physicochemical parameters are summarized in table 1. The extractive values were used to find out the amount of active principles. The higher percentage yield of C.tora was found to be 15.68 ± 2.67 in water extract (table 1). This showed that leaves of C.tora contain higher concentration of polar constituents. The ash values were used to detect the presence of any foreign matters such as sand and soil, water soluble salts adhering to the surface of the drugs. There is always a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials. The water-soluble ash was used for the measurement of inorganic elements. The total ash values of a plant drugs are not always trustworthy due to the possibility of presence of non-physiological substances. So, the authentication of acid insoluble ash was also done which showed lowest content of acid insoluble ash in the extract of the leaves (table 1).

Table 1: Physicochemical analysis of C.tora extracts (n=3).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maceration extractive values</td>
<td>1.14 ± 0.12</td>
<td>4.87 ± 0.54</td>
<td>8.56 ± 0.67</td>
<td>9.87 ± 0.84</td>
</tr>
<tr>
<td>2</td>
<td>Hot extractive values</td>
<td>1.43 ± 0.14</td>
<td>6.65 ± 0.45</td>
<td>12.87 ± 1.43</td>
<td></td>
</tr>
</tbody>
</table>
3.2. Preliminary phytochemical analysis

The leaves extracts of *C. tora* in different solvents were screened for the presence of major constituents. The preliminary phytochemicals screening revealed the presence of flavonoids, alkaloids, carbohydrates, phenolic compounds, saponins, steroids, tannins and proteins etc.

The methanolic extracts showed the presence of all above constituents except lipid contents. The results of phytochemicals screening are summarized in table 2.

Table 2: Phytochemical analysis of *C. tora* extracts.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Pet ether</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipids/fats</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3.3. Total phenolic contents

The total phenolic contents of methanolic extract were measured by UV spectrophotometric method. The total content of phenolic compounds was found to be $10.67 \pm 1.46$ mg/gm in methanolic extract of *C. tora* leaves. The presented values are Mean ± SEM. of triplicate determinations. The total phenolic content was expressed as gallic acid equivalent in mg/gm of the extract. The standard calibration curve was used to calculate the total phenolic contents in the methanolic extract of the leaves (Fig. 2).
3.4. **Total flavonoids contents**

The flavonoids content was determined by UV spectrophotometric method. The total content of flavonoids was found to be 1.86 ± 0.45 mg/gm in methanolic extract of leave. The presented values are Mean ± SEM of triplicate determinations. The total flavonoids contents in the sample extract were calculated by using standard calibration curve (Fig. 3). The total flavonoid content was expressed as rutin equivalent in mg/gm of the extract. The phenolic and flavonoid compounds act as antioxidants. These compounds are also reported to have anticancer, antimicrobial, anti-inflammatory and anti-allergic activities etc. Phenolic compounds are most widely occurring groups of phytochemicals. These compounds are secondary metabolites which have vital role in reproduction and growth, gives protection against harmful predators and pathogens (Eleazu et al., 2012).

3.5. **Determination of heavy metal residues**

Lead, cadmium, arsenic and mercury are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and
herbal ingredients. (Mukherjee, 2002). Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration. Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, hemorrhagic injuries, anemia and cardiovascular disorders (Kokoski et al., 1958). Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity. The determination of heavy metals (Arsenic, Mercury Cadmium and Lead,) analysis was carried out in the extracts of leaves of the C.tora on atomic absorption spectrophotometer. All necessary safety measures were adopted to avoid any possible contamination of the sample as per the AOAC guidelines. Cadmium was found to be 0.28 ± 0.04 mg/kg. It was below the acceptable limit of 0.3 mg/kg as prescribed by WHO. Lead was found to be 0.48 ± 0.08 mg/kg which was far below the acceptable limit of 10 mg/kg as prescribed by WHO. Arsenic and Mercury were detected in all samples. Both metals were found to be within acceptable limits (Table 3).

Table 3: Determination of toxic heavy metals in C.tora extracts (n=3).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Heavy metal</th>
<th>Concentration (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arsenic (As)</td>
<td>0.54 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium (Cd)</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>Lead (Pb)</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>Mercury (Hg)</td>
<td>0.32 ± 0.09</td>
</tr>
</tbody>
</table>

3.6. Determination of pesticide residues

Pesticides residues were determined in the extracts by GC-MS according to AOAC guidelines (Anonymous, 2006). Total 15 pesticides were tested in all the samples, none of the pesticides was found in the C.tora extract. Pesticides are the toxic substances; the drugs should be free from these substances. (Anonymous 2002; Anonymous 2003). Results are presented in table 4.

Table 4: Determination of pesticide residue in C.tora extracts.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pesticide</th>
<th>Test method</th>
<th>Results</th>
<th>MDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-BHC</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>β-BHC</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>γ-BHC(Lindaneel)</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>δ-BHC</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>Heptachlor</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>6</td>
<td>Heptachlor_Epoxide</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>α-Chlordane</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
<tr>
<td>8</td>
<td>α-Endoulfan</td>
<td>AOAC970.52/EPA525.5</td>
<td>Not detected</td>
<td>0.01mg/kg</td>
</tr>
</tbody>
</table>
3.7. Aflatoxin analysis

Aflatoxins were analyzed in the different plant extracts by HPLC method as described by AOAC method 980.20- ITEM-I (Scott, 1990). Different aflatoxins like B₁, B₂, G₁ and G₂ were determined in the leaves extract. No aflatoxin was detected in the sample of the extract of the drug. Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including humans. Toxigenic fungi may contaminate herbal products at different phases of production and processing, mainly in favorable humidity and temperature conditions. Many mycotoxins also have significant chemical stability, which enables their persistence in products even after the removal of the fungi by means of the usual manufacturing and packaging processes. The most common toxigenic fungi found in plants include species from the genera Aspergillus and Fusarium. Fusarium verticillioides. Aspergillus niger produce aflatoxins B₁, B₂, G₁ and G₂ which are considered to be involved in the etiology of human liver cancer. (Willem et al 2001). Results are summarized in table 5

Table 5: Determination of aflatoxin residue in C.tora extracts.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Aflatoxin</th>
<th>Test method</th>
<th>Results</th>
<th>MDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B₁</td>
<td>AOAC 990.332</td>
<td>Not detected</td>
<td>1.0 µg/kg</td>
</tr>
<tr>
<td>2</td>
<td>B₂</td>
<td>AOAC 990.332</td>
<td>Not detected</td>
<td>1.0 µg/kg</td>
</tr>
<tr>
<td>3</td>
<td>G₁</td>
<td>AOAC 990.332</td>
<td>Not detected</td>
<td>1.0 µg/kg</td>
</tr>
<tr>
<td>4</td>
<td>G₂</td>
<td>AOAC 990.332</td>
<td>Not detected</td>
<td>1.0 µg/kg</td>
</tr>
</tbody>
</table>

CONCLUSION

The generated scientific data of this study might be used to establish its quality standards and may be utilised to develop pharmacopoeial monograph of C. tora L. leaves. The outcome of this study might prove beneficial in herbal industries and academic institutes for identification, purification and standardization of C. tora L. leaves.

Conflict of interest

The authors declare no conflicts of interest.
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REFERENCES


