Pharmacognostical screening of seeds of Cassia absus

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ABSTRACT
Cassia absus is considered to be useful in many ailments and diseases. It is traditionally used for many medicinal purposes. The seeds have a bitter, bad taste and having diuretic, cathartic and useful in the liver and kidney diseases. It is very helpful in reducing the swelling and is effective in eye ailments. It also prevents hemorrhages. Its alkaline nature helps in scrapping of the extended skin. The seeds are also having astringent properties. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Morphological and anatomical aspects as well as differential micro chemical response have been worked out to identify the diagnostic features of the leaf. Physical constant values involving moisture content, ash and extractives as well as qualitative and quantitative estimation of various phytochemicals have been studied. The presence of lipid, saponin, tannin, alkaloid, phenol, steroid, flavonoid, and some other chemical constituents are recorded.

INTRODUCTION
Cassia absus (Family: Fabaceae) is commonly known as “Jasmeejaz” in English and “Chimed” in “Hindi” also used in India. It grows as a sticky plant in almost all the states of India particularly North-West India. The plant is mainly useful in the skin diseases and eye ailments. The seeds have a bitter, bad taste and having diuretic, cathartic and useful in the liver and kidney diseases. The seeds are used as astringent and cathartic properties. It is very helpful in reducing the swelling and is effective in eye ailments. It also prevents hemorrhages. However, no scientific standards or pharmacognostical parameters are yet available to ascertain the identity and to determine the quality of the crude drug. The pharmacognostical parameters are the major and reliable criteria for the conformation and identity and determination of quality and purity of the crude drug.

The present work therefore attempts to report various necessary pharmacognostic and phytochemical standards of Cassia absus.

MATERIALS AND METHODS

Plant Material
The seeds of Cassia absus Linn. was procured from a local vendor in Mumbai in the month of January-2010.

The seeds were verified by a well known taxonomist and deposited in the Department of pharmaceutical chemistry, SVKM’s NMIMS University, Mumbai for further reference. Collected fresh seeds were washed and used for the study of macroscopic and microscopic characteristics. The dried seeds of the plant was powdered and passed through 40 mesh size and stored in an airtight container for further use.

Reagents and Chemicals
All reagents and chemicals used for testing were analytical grade obtained from Fisher Chemicals Ltd., Mumbai and Loba chemie, Mumbai.

Organoleptic Evaluation
The freshly collected seeds were spreaded on a dry plastic sheet and investigated different organoleptic features by repeated observations using magnifying glass and ruler and then recorded.

Macroscopic study
The macroscopic characters of the seeds of Cassia absus were studied and reported in the results.

Microscopic study of powdered plant material
1 gm of powder was boiled with Chloral Hydrate solution for about 5 minutes to remove chlorophyll and
any other fatty impurity. Then, powder was placed on
the grease free microscopic slide along with the drop
of Glycerin: water (1:1). The powder was covered with
clean cover slip and observed under the compound
microscope at 40X magnification. A camera lucida
was attached with the microscope and the powder was
suitably traced out.

Physicochemical evaluation

Physicochemical properties such as the percentage
of loss on drying (LOD), Total ash, Acid insoluble ash,
Water soluble ash, were determined according to the
specifications as per Indian Pharmacopeia\(^5\). Water and
alcohol soluble extractive values were estimated by cold
maceration according to the method as prescribed by
WHO\(^6\).

Phytochemical screening (7, 8)

The powdered seeds were subjected to preliminary
Phytochemical screening for qualitative detection of
phytoconstituents. The dried and coarsely powder seeds
(500 g) were extracted successively with petroleum ether
\((40^\circ \text{C} - 60^\circ \text{C})\), Chloroform \((59.5^\circ \text{C} - 60^\circ \text{C})\), Ethyl
acetate \((76.5^\circ \text{C} - 77.5^\circ \text{C})\), Methanol \((64.5^\circ \text{C} - 65.5^\circ
\text{C})\), Hydro alcoholic mixture (Methanol:water-1:1) and
water successively with continuous cold maceration.
Each time before extracting with the next solvent of
high polarity, the powdered drug (marc) was dried in
air oven below 50\(^\circ\) C for 10 minutes. Each extract was
concentrated by distilling off the solvent, which was
recovered subsequently. The concentrated extracts were
evaporated to dryness and the extract obtained with each
solvent were weighed. Their percentages were calculated
in terms of initial air dried plant material. The colors of
the extracts were observed. The successive extracts as
mentioned above, were subjected to various qualitative
phytochemical tests for the identification of chemical
constituents present in the plant material.

Thin Layer Chromatography study(9)

Accurately weighed 100 mg methanolic extract
was diluted with 5 ml of methanol in a clean glass
stopped volumetric flask and used for spotting the
chromatographic plates. Silica Gel 60 F Plates were used
as a stationary phase. Mobile phase was Butanol: Acetic
acid: Water \((4:1:1)\). After development of TLC plates
by one dimensional ascending method\(^9\), visualization
was performed by spraying the reagent. The \(R_f\) values
were recorded carefully and the chromatogram was
documented graphically\(^10\).

Fluorescence Analysis: (11, 12)

A small quantity of dried powdered seeds was placed on a
grease free clean microscope slide and added 1-2 drops of
freshly prepared reagent solution, mixed by gentle tilting
the slide and waited for 12 minutes. Then, the slide was
placed inside the UV chamber and viewed in day light,
short (254 nm) and long (366 nm) ultraviolet radiations.
The colors obtained by application of different reagents
in different radiations were recorded.

RESULTS

Macroscopy

Diameter - Most of the seeds are around 1-1.2 cm in diameter.
Shape - Mostly circular and sometimes uneven in texture

Organoleptic Characters

Color - Externally black and internally yellow
Taste - Bitter
Odour - Odourless

Extra features

Testa is hard smooth and glossy in appearance.

Microscopic study of powdered drug:

Powder of seeds mainly shows wavy sclerenchyma and
pigment layer.

Endosperm

Polygonal cells are present with cellulose like material
inside the cells.

Epidermis

Polygonal epidermal cells, filled with mucilage are present.

Hypodermis

Rounded collenchymatous cells are present.

Trichomes

Entire or fragments of trichomes are present.

Wavy sclerenchyma

Special rounded wavy sclerenchymatous cells are present.

Pigment Layer

Fragments of pigment layer, square cells with yellow to
orange mass are Present.
Table I: Results of phytochemical screenings of successive fresh extracts of Cassia absus seeds

<table>
<thead>
<tr>
<th>Extracts Used</th>
<th>P. Eth (60–80°C)</th>
<th>Ethyl acetate</th>
<th>CHCl₃</th>
<th>Methanol</th>
<th>Hydro alcoholic</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>Fixed oils and fats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phenolic compounds and tannins</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>Proteins and amino acids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>Gums and mucilages</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>Volatile oil</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>Flavanoids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present, − = Present

Table II: Results of Physicochemical properties

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Physicochemical properties</th>
<th>Result (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ash Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total ash</td>
<td>4.26</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Sulphated ash</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>Foreign Organic Matter</td>
<td>0.025</td>
</tr>
<tr>
<td>3</td>
<td>Moisture content (Loss On Drying)</td>
<td>1.71</td>
</tr>
<tr>
<td>4</td>
<td>Swelling Index</td>
<td>134.56</td>
</tr>
<tr>
<td>5</td>
<td>Mucilage Content</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Table III: Fluorescence analysis of powdered Cassia absus Linn. seeds

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Treatment</th>
<th>Day Light</th>
<th>UV (254 nm)</th>
<th>UV (366 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Yellow Brown</td>
<td>Dark Brown</td>
<td>Yellow Brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1 M NaOH</td>
<td>Yeellowish Brown</td>
<td>Light Brown</td>
<td>Black Brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + Picric acid</td>
<td>Bright Yellow</td>
<td>Greenish Brown</td>
<td>Cream Yellow</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 1 M HCl</td>
<td>Cream Brown</td>
<td>Greenish Black</td>
<td>Brown</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1 M Acetic Acid</td>
<td>Cream Brown</td>
<td>Dark Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>6</td>
<td>Powder + dilute HNO₃</td>
<td>Orange Brown</td>
<td>Green Brown</td>
<td>Buff Brown</td>
</tr>
<tr>
<td>7</td>
<td>Powder + 5% FeCl₃</td>
<td>DarkYellow Brown</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>8</td>
<td>Powder + dilute NH₃</td>
<td>Floroscent Yellow</td>
<td>Black</td>
<td>Green Yellow</td>
</tr>
<tr>
<td>9</td>
<td>Powder + Methanol</td>
<td>Yellow Brown</td>
<td>Buff Brown</td>
<td>Black Brown</td>
</tr>
<tr>
<td>10</td>
<td>Powder + 50 % HNO₃</td>
<td>Orange Brown</td>
<td>Green Brown</td>
<td>Buff Brown</td>
</tr>
<tr>
<td>11</td>
<td>Powder +NH₃ + HNO₃</td>
<td>Yellowish White</td>
<td>Blackish White</td>
<td>Buff Brown</td>
</tr>
<tr>
<td>12</td>
<td>Powder + 1 M H₂SO₄</td>
<td>Dark Brown</td>
<td>Greenish Brown</td>
<td>Floroscent White</td>
</tr>
<tr>
<td>13</td>
<td>Powder +Dilute I₂</td>
<td>Brownish Red</td>
<td>Blackish Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>14</td>
<td>Powder + K₂Cr₂O₇</td>
<td>Orange</td>
<td>Greenish Brown</td>
<td>Brownish Orange</td>
</tr>
<tr>
<td>15</td>
<td>Powder + Methanol</td>
<td>Brown</td>
<td>Blackish Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>16</td>
<td>Powder + Toluene</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
<td>Yellowish Brown</td>
</tr>
<tr>
<td>17</td>
<td>Powder + KOH</td>
<td>White Brown</td>
<td>Dark Brown</td>
<td>Brown</td>
</tr>
</tbody>
</table>

Figure 7: Comparative TLC profiles of all 6 Extracts
Pharmacognostical screening of seeds of Cassia absus

**Phytochemical Screening**

The results are shown in the Table (1). The results demonstrated the presence of phytosterols, Tannins and phenolic compounds and Flavanoids in the dried seeds of Cassia absus.

**Physico-chemical Evaluations**

The values of all the determinations are summarized in the Table (2). Water soluble ash is somewhat higher than the water insoluble ash. Water and methanolic extracts are having almost same extractive values.

**Thin Layer chromatography Results**

The results for TLC are shown in the figure (7).

**Fluorescence analysis**

The results summarized in the Table (3).

**Determination of Inorganic elements in ash**

Aluminum, Calcium, Chlorides, Phosphates and Potassium are present in ash of the seeds. The results are summarized in the Table (4).

<table>
<thead>
<tr>
<th>Elements present in ash</th>
<th>Elements absent in ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>Copper</td>
</tr>
<tr>
<td>Chlorides</td>
<td>Carbonates &amp; bicarbonates</td>
</tr>
<tr>
<td>Calcium</td>
<td>Iron</td>
</tr>
<tr>
<td>Phosphates</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Potassium</td>
<td>Nitrates</td>
</tr>
<tr>
<td>Sulphates</td>
<td>Zinc</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
</tr>
</tbody>
</table>

**DISCUSSION**

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years, there has been an emphasis on standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO), the microscopic and macroscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

The organoleptic, macroscopic and microscopic studies yielded important characteristics. The present study was focused on the structural features of seeds of *Cassia absus* including micro and macroscopic features and physicochemical properties of grinded seeds. The microscopic characters revealed the presence of wavy sclerenchyma and pigment layer. These characteristics might be useful for distinguishing it from its substitutes and adulterants.

On the basis of qualitative chemical test, it has been observed that chemically therapeutic compounds like Tannins and phenolic compounds, Flavanoids, and saponin glycosides were present in sufficient amount in the seeds of *Cassia absus*. On the basis of elemental analysis, it has been revealed that Aluminum, Calcium, Chlorides, Phosphates and Potassium are present in rich amount. The extracts were subjected to qualitative phytochemicl tests to find out the active constituents. In the fresh seeds extract of *Cassia absus* with chloroform, ethyl acetate, methanol, hydroalcoholic and water saponin glycosides, Flavanoids and Tannins and Phenolic compounds were present.

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in ash residues consisting of an inorganic material (metallic salts and silica). These parameters were used for the determination of inorganic materials like phosphates, aluminum, calcium, and sodium. Heating causes the loss of organic material in the form of CO$_2$ leaving behind the inorganic components. We can detect the extent of adulterants as well as establish the quality and purity of the drug by using this method. The ash value determines the quality of the drug material. Here, the total ash value obtained was around 4 percent. Acid insoluble ash was 0.72 percent which determines the acid insoluble component present in the ash and water soluble ash was 1.02 percent which was the water soluble fraction of the total ash.

The extraction of any crude drug with particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The extractive value of the crude drug determines the quality as well as purity of the drug material. Methanol and water extractive values were 3.0 and 4.2 percent respectively. The use of a single solvent
can be the means of providing preliminary information on the quality of a particular drug sample. The extractive value of the crude drug determines the quality as well as purity of the drug material. The loss on drying value was found to be 1.71% w/w. It signifies the considerable amount of moisture in seed materials. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and also be controlled to make the solution of definite strength. The moisture content of a drug should be minimised in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination. The objective of drying of fresh material is to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug and to reduce their weight and bulk. Not only is the ultimate dryness of the crude drug is important, equally important is the rate at which the moisture is removed and the conditions under which it is removed. If the rate is too slow, much spoilage may occur before the drying process is completed. Therefore, in general, drying should be accomplished as rapidly as is possible with good practices.

Thin layer chromatograms are produced with the aim of identifying the individual substances in a mixture and also for testing for purity or for separation of mixtures. They are particularly useful for checking the mixtures used for synthetic reactions or following the course of reactions. For purposes of identification, it is necessary to relate the Rf values of the investigated substances and those of reference substances. If the Rf value agree, it is probable but not certain, that the two spots correspond to the same substance. Reliable identification is only possible by using spectroscopic investigation alongside with thin layer chromatography.

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. The powder drugs exhibit different fluorescence character in the presence of different chemical reagents under ultra-violet light. The change in the colour of stem powder under UV radiation in reference to day light was observed. The powder drug exhibit different fluorescence character due to presence of different functional groups in drug chemical constituents. The above table is about the fluorescence characteristics of seed powder of Cassia absus Linn. in the presence of different chemical reagents and ultra-violet light at 254nm and 365nm respectively.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. [12,16]

Total phenolic content was determined using Folin – Ciocalteu reagent which was found to be 3.39 % w/w and total tannin content was found to be 0.76% w/w using Folin – Denis reagent.

CONCLUSION:

After present investigation it can be concluded that the pharmacognostical study of Cassia absus Linn. seeds yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. As previously mentioned, Cassia absus Linn. being a morphologically variable species, these information will also be helpful to differentiate Cassia absus Linn. from the closely related other species and varieties of Cassia.

Figures: Results for Microscopic study

![Figure 1: Endosperm](image)
Pharmacognostical screening of seeds of Cassia absus

Figure 2: Epidermis

Figure 3: Trichomes

Figure 4: Ca-Oxlate crystals

Figure 5: Wavy sclerenchyma
Pharmacognostical screening of seeds of Cassia absus

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