PHARMACOGNOSTIC STANDARDIZATION AND HPTLC FINGERPRINT OF CRATAEVA TAPIA L. SEEDS

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ABSTRACT

Plants have been the basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Plants are one of the richest sources of bioactive compounds. *Crataeva tapia* Linn. ssp. *odora* (Jacob.) Almedia (syn. *C. religiosa* var. *nurvula* Hook. f.) belonging to the Family- Capparaceae is commonly called as Varuna. The plant parts (bark, leaves and seeds) have many medicinal uses. Fruits of the plant are used as tonic and febrifuge in Northeast of Brazil. Pentadecane, octanamide, 12-tricosanone, friedelin, ceryl alcohol, tricontate, tricontanol, β-sitosterol and glucoapparin have also been isolated from fruits. Pharmacognostic studies have been carried out for bark and leaf however, inadequate data exists for seeds. The present investigation was carried out to study pharmacognostic characters, preliminary phytochemical analysis and HPTLC fingerprint profile of *Crataeva tapia* L. seeds. In the present study, water soluble extractive value was found to be more than alcohol soluble extractive value. Preliminary phytochemical analysis revealed the presence of starch, aleurone grains, amino acids, carbohydrates, fats and fixed oils, alkaloids, steroids, glycosides, tannins and triterpenoids. HPTLC fingerprint profile developed would serve as a tool for identification and quality control of the seed. The present study will thus play a vital role in identification, authentication and maintaining proper quality standards of *Crataeva tapia* L seeds.

KEYWORDS: *Crataeva tapia* L., seeds, pharmacognosy, HPTLC fingerprint profile.
INTRODUCTION
Healing with medicinal plants is as old as mankind itself. Awareness of medicinal plants usage is a result of struggle against illnesses due to which man learned to pursue drugs in barks, seeds, fruits and other parts of the plants. Contemporary science has acknowledged their active action and it has included in modern pharmacotherapy, a range of drugs of plant origin known by ancient civilizations and used it throughout the millennia. [1]

A key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and non-compliance of GMP guidelines basically due to poor standardization status. It is very important that a system of standardization be established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. Due to natural heterogeneity, plant material may vary in its phytochemical content and therefore in its therapeutic effect according to different places and time of collection. Adding to this variability it is the fact that in herbal medicine several plants may be used together in the same preparation. These factors substantiate basic need of standardized quality control tests for herbal preparations to ensure quality of the product. [2]

Crataeva tapia Linn. ssp. odora (Jacob.) Almedia (syn. C. religiosa var. nurvula Hook. f.) (henceforth written as Crataeva tapia L.) belonging to family Capparaceae is a moderate, much branched deciduous tree, commonly called as ‘Varuna’ (Plate No: 1a). Crataeva tapia L. is globally distributed in India, Myanmar, Indonesia and China. In India it is found in Peninsular India, Western India, Gangetic Plains and Eastern India upto Tripura and Manipur. [3] It is often found along streams, but also in dry deep boulder formations in sub-Himalayan tract. It is usually cultivated in vicinity of temples in Central India, Bengal and Assam. [4]

have also been isolated from fruits \cite{20}. Pharmacognostic studies have been carried out for bark and leaf however, inadequate data exists for seeds.

Thus in the present work, a detailed pharmacognostic study, phytochemical analysis and HPTLC fingerprint profile of *Crataeva tapia* L. seed is carried out which will further help in crude drug identification as well as in standardization. Pharmacognostic study carried out includes; determination of macroscopical, microscopical characteristics, physicochemical constants, fluorescence analysis, preliminary phytochemical analysis and HPTLC fingerprint profile.

**MATERIALS AND METHODS**

The flowering twig of *Crataeva tapia* L. was collected from Kalyan M.S., India. Herbarium was prepared and authenticated from Blatter Herbarium, St. Xavier’s College, Mumbai. The seeds were collected from fully ripe fruits with crimson colored skin in the month of February (plate 2d and 2e). Fruits were opened; seeds were scooped out and washed under running tap water and rubbed against sieve to remove any adhering pulp. The seeds were air dried, ground to powder and stored in a clean, dry, air tight container till further use.

**Pharmacognostic studies**

**Macroscopy:** Macroscopic or morphological description including size, shape and organoleptic characteristics were determined according to methods given by Mukherjee \cite{21}.

**Microscopy:** Transverse section of the seed and powder microscopy of seed were carried out according to methods outlined by Khandelwal \cite{22}.

**Physico chemical constants:** Physico chemical constants such as percentage of total ash, water soluble and acid insoluble ash, water and alcohol soluble extractive values were calculated according to methods described in Indian Pharmacopoeia \cite{23}.

**Preliminary phytochemical analysis:** Phytochemical analysis was performed as described by Khandelwal \cite{22} and Kokate \cite{24}. 1 gm of the powder was soaked overnight in 10 ml of solvent *viz.*, Petroleum ether, Chloroform, Methanol, Ethanol and Water respectively and tested for the presence of phytoconstituents.

**Fluorescence analysis:** It was carried out using the method given by Kokoski \cite{25} and Chase and Pratt \cite{26}. Small quantity of seed powder was placed on a grease free slide. It was treated
with 1-2 drops of freshly prepared reagents (Table 2), examined under short UV (254 nm), long UV (366 nm), visible light and the change in colour of the plant powder was noted down.

**HPTLC fingerprint profile:** A qualitative densitometric HPTLC analysis was performed with methanolic extract for the development of characteristic fingerprint profile, which may be used for quality evaluation and standardization of the drug. 25 µl of the methanolic extract of *C. tapia* L. seed powder was loaded on precoated silica plate (silica gel G60 F254 - Merck) using CAMAG LINOMAT V applicator. The plate was developed in CAMAG twin trough chamber (10 X 10 cm) presaturated for 20 minutes with mobile phase Toluene: Ethyl acetate: Glacial acetic acid (6:3:0.1 v/v). The plate was further derivatized using Anisaldehyde Sulphuric acid, visualized using CAMAG TLC visualizer and scanned densitometrically using CAMAG TLC Scanner 3 (winCATS software).

**RESULTS**

**Macroscopy**

Fruits are red when ripe, globose, many seeded ovoid berry. Seeds are embedded in yellow pulp of the fruit. Seeds are hard, sub-spherical to orbicular (circular or spherical), dorsiventrally flattened (having distinct upper and lower surfaces), about 0.5 mm in diameter, 0.2 mm in thickness. A narrow deep groove runs eccentrically in the central region of the seed (deviating from normal). (Plate No: 1d, 1e, 1f, 1g)

**Organoleptic Characters of seed powder**

<table>
<thead>
<tr>
<th>Character</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Brown</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine sticky powder</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter</td>
</tr>
</tbody>
</table>

**Microscopy**

**T.S. of Seed**

T.S. of seed shows an outer layer of arcuatly running epidermis of testa filled with brownish contents and covered with cuticle, followed by 1-2 layers of radially arranged, thick walled, narrow lumened palisade like sclerenchymatous cells of different sizes embedded at places with dark brown contents. Underneath this lies 4-5 layers of collapsed parenchymatous cells filled with brownish contents, followed by a layer of small sized rectangular, tangentially running epidermal cells of endosperm which is very wide and composed of spherical, thin
walled parenchymatous cells. These parenchymatous cells are embedded with aleurone grains and oil globules (Plate No: 1h).

**Powder microscopy of seed powder**

Powder microscopy revealed the presence of fragments of overlapping parenchymatous cells of collapsed layers of testa embedded with brown colored pigments in surface view, fragments of endospermic cells showing presence of oil globules and sclereid (Plate No: 1i, 1j, 1k).

**Physico chemical constants**

Ash values of a drug gives an estimate of the inorganic components (metallic salts and silica). The percentage of total ash, acid insoluble ash and water soluble ash were found to be 3.11, 0.19 and 1.74% respectively. The extractive values of the seed for water and alcohol were found to be 17.89 and 9.3% respectively (Table No. 1).

**Flourescence analysis**

The change in colour of the seed powder on treating with respective reagent is presented in Table No. 2.

**Preliminary Phytochemical Analysis**

Preliminary phytochemical analysis revealed the presence of starch, aleurone grains, alkaloids, amino acids, steroids, carbohydrates, fats and fixed oils, glycosides, tannins as presented in Table No. 3.

**HPTLC**

HPTLC fingerprint profile of methanolic extract of seed powder showed distinct band pattern after spraying with derivatizing reagent anisaldehyde sulphuric acid. Rf values under different wavelengths after derivatization are tabulated in Plate No. 2.
Plate No. 1: *Crataeva tapia* L.


Plate No. 2: HPTLC fingerprint profile of methanolic extract of *Crataeva tapia* L. seed

At 366 nm
At 550 nm

**Table No. 1: Physico-chemical constants of *Crataeva tapia* L. seeds**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ash value (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total ash</td>
<td>3.11±0.19</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>0.19±0.09</td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>1.74±0.14</td>
</tr>
<tr>
<td>2.</td>
<td>Extractive values (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble</td>
<td>17.89±1.5</td>
</tr>
<tr>
<td></td>
<td>Alcohol soluble</td>
<td>9.3±1.9</td>
</tr>
</tbody>
</table>

Values are mean of three sets of determinants

**Table No 2: Fluorescence analysis of *Crataeva tapia* L. seeds**

<table>
<thead>
<tr>
<th>No.</th>
<th>Tests</th>
<th>Visible</th>
<th>UV (254 nm)</th>
<th>UV (366nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Light Brown</td>
<td>Buff brown</td>
<td>White Brown</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + 1N NaOH in Methanol</td>
<td>Dark Brown</td>
<td>Black Brown</td>
<td>White Brown</td>
</tr>
<tr>
<td>5.</td>
<td>Powder + 1 N HCl</td>
<td>Brown</td>
<td>Brown</td>
<td>Orange</td>
</tr>
<tr>
<td>7.</td>
<td>Powder + 1N NaOH</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>8.</td>
<td>Powder + 1N NaOH + Nitrocellulose in Amyl acetate.</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>9.</td>
<td>Powder + HNO₃ (1:1)</td>
<td>Brown</td>
<td>Dark Brown</td>
<td>Yellow</td>
</tr>
<tr>
<td>10.</td>
<td>Powder + H₂SO₄ (1:1)</td>
<td>Brown</td>
<td>Dark Brown</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
Table No 3: Preliminary phytochemical analysis of *Crataeva tapia* L. seed extracts

<table>
<thead>
<tr>
<th>No.</th>
<th>Tests for Phytoconstituents</th>
<th>PE</th>
<th>CE</th>
<th>ME</th>
<th>EE</th>
<th>AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aluerone grains</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Amino Acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Fats and Fixed Oils</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Starch</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6.</td>
<td>Protein</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Alkaloids</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Anthraquinones</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9.</td>
<td>Flavonoids</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Glycosides</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>11.</td>
<td>Saponins</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12.</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13.</td>
<td>Tannins</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>Triterpenoids</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

PE = Petroleum Extract,  
CE = Chloroform Extract,  
ME = Methanolic Extract,  
EE = Ethanolic Extract,  
AE = Aqueous Extract,  
ND = Not Detected.

**DISCUSSIONS**

Over the past decades, herbal medicine has become an interest of global significance with medicinal and economic implications. Wide spread use of herbs throughout the globe has raised serious concerns over its quality, safety and efficacy. Thus, exact scientific assessment has become a precondition for acceptance of herbal health claims\(^\text{[27]}\).

Quality control standardizations of various medicinal plants used in traditional medicine is becoming more important today in view of the commercialization of formulations based on these plants \(^\text{[28]}\). Pharmacognostic study is the preliminary step in standardization of crude drug. Physico-chemical parameters like ash value reveals the presence of inorganic radicals like phosphate, carbonate and silicates. This will help in evaluating the purity of drugs i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Data of extractive value shows that the amount of water soluble phytoconstituents is more than alcohol soluble phytoconstituents in seed.

The macroscopic or morphological description of a crude drug includes size, shape, nature of outer and inner surfaces and organoleptic characteristic\(^\text{[24]}\). Organoleptic evaluation of drug
refers to the evaluation of a drug by color, odour, size, shape, taste and special features including touch, texture etc. Since the majority of information on the identity, purity and quality of the material can be drawn from these observations, they are of primary importance before any further testing can be carried out. Organoleptic evaluations can be done by means of organs of sense which includes the above parameters and thereby define some specific characteristic of the material which can be considered as a first step towards establishment of identity and degree of purity. The color is of use in indicating the general origin of the drug eg. material derived from the aerial part of the plant is usually green and the underground plant material is usually devoid of green color \(^{[21]}\). *Crataeva tapia* L. seeds are brown in color, fine oily powder having bitter taste.

Quality control of herbal drugs has traditionally been based on appearance, but today microscopic evaluation is indispensable in the initial identification of herbs, small fragments of crude or powdered herbs and detection of foreign matter and adulterants. A primary visual evaluation, which seldom needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used \(^{[29]}\). Thus, macroscopic and microscopic studies of *Crataeva tapia* L. seeds will help in its proper identification and collection.

Fluorescence is the phenomenon exhibited by various drugs and reveals various chromophores of chemical constituents present in the plant material. Some constituents show fluorescence in the visible range daylight. UV light produces fluorescence indicative of many natural products (Eg: Alkaloids like Berberine), which do not visibly fluoresce in daylight. If the substance 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 by this method and also it is an important parameter of pharmacognostical evaluation \(^{[30]}\). Fluorescence analysis will thus help to detect any adulterants present in seed powder of *Crataeva tapia* L.

Morphological authentication is not sufficient to ensure quantitative consistency of bioactive or marker compounds responsible for the therapeutic effects. Advances in chemical and
instrumental techniques have made it easier to estimate phytochemical parameters of crude drugs [31]. Chromatographic fingerprint have been suggested to check for authenticity or provide quality control of herbal medicine. Chromatography has the advantage of separating a complicated system into relatively simple sub-systems and then presenting the chemical patterns of herbal medicine in the form of a chromatogram [32-35]. HPTLC fingerprint profile would serve as a tool for identification, authentication and quality control of herbal drugs.

Fingerprint analysis approach using chromatography (TLC, GC and HPLC) have become the most potent tool for quality control of herbal medicines because of its simplicity and reliability [36]. A simple chromatographic technique such as TLC may provide valuable additional information to establish the identity of the plant material. This is especially important for those species that contain different active constituents. It is a powerful and relatively rapid solution to distinguish between chemical classes, where macroscopy and microscopy will fail. Chromatograms of essential oils, for example, are widely published in the scientific literature, and can be of invaluable help in identification [37]. HPTLC fingerprint profile along with their Rf values and percentage proportion were recorded, which will help in identification and quality control of Crataeva tapia L. seeds.

CONCLUSION
To ensure reproducible quality of plant herbal products, proper control of starting material is of utmost essential, thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. There are number of crude drugs where the plant source has not yet been scientifically identified. Herbal medicines can be relevant today only if they are applied and tested within framework of modern science and subjected to most rigorous criteria of quality, safety and efficacy. Pharmacognostic study gives the scientific information regarding the purity, quality and is the preliminary step in the standardization of crude drugs. Pharmacognostic evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. HPTLC fingerprint profile would serve as a tool for identification and quality control of the seed. The detailed study of Crataeva tapia seeds will thus help in standardization.

REFERENCES


