EXTRACTION AND ITS PHARMACOGNOSTICAL STUDY OF BARK OF CRATAEVA RELIGIOSA

*Dr. P. Gowsalya
Assistant Professor and Head, Dept. of Biochemistry, Navarasam Arts and Science College for Women, Arachalur, Erode(Dt).

ABSTRACT
The Crataeva religiosa (Hook and Frost) is one of the herbal drug, belongs to the family capparidaceae. The name Crataeva is given in the honor of Crataevus, a Greek botanist, who was living in the time of Hippocrates and the name religiosa indicates its growth near the places of worship. The drug is well known for its various pharmacological properties like diuretic, antiinflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithonotriptic, antihemarthic, antiperiodic, antomyctic, contraceptive, antipyretic, antithelmintic, rubifacient and vasicant properties. The bark of the Crataeva religiosa is useful in the urinary disorders and kidney stone remover. The various pharmacognostical studies such as microscopy, macroscopy ash value and extractive values reveals that the identity of genuine drug source.

KEYWORDS: Crataeva religiosa, Pharmacognostical, macroscopy, microscopy.

INTRODUCTION
Indian traditional system of medicine is based on pragmatic facts of the observations and the experience over millennia. Traditional medicine, being a significant element in the cultural patrimony, still remains the main choice for a large majority of people for treating various diseases. The WHO has estimated that 80% of the population of the developing countries depends on traditional medicine mostly derived from plants for their primary health care needs. The demand of medicinal plants is increasing throughout the world. 90% of the drugs used in Indian systems of medicine and Homeopathy are plants based and collected from wild source. In recent years traditional drugs are receiving great attention all over the world; therefore a great emphasis has been laid to revive the heritage knowledge on the medicinal plants. Medicinal plants have been man’s oldest friends in his efforts at health and healing. Hence the present study is focused to evaluate all the traditional information gathered through various literatures has been taken into our account to valuate in scientific manner.

MATERIALS AND METHODS
Macroscopic Analysis
The bark is green, smooth and soft. The bark surface exhibits narrow and shallow tissues. The bark is fibrous in texture. The bark has no specific odour or taste.

Microscopical Studies: Fixing and killing
The bark were cut and removed from the plant and fixed in FAA (Farmalin- 5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary–butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning
The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O’Brien et al. (1964). Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI(for Starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid (Sass,
were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Naoh and mounted in glycerine medium after staining. Different cell component were studied and measured.

**Photomicrographs**

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with NIKON ALPHA PHOTO -2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features were taken from the standard Anatomy books (Esau, 1964).

- Determination of Total Ash (Indian Pharmacopoeia, 1996)
- Determination of Sulphated Ash (Indian Pharmacopoeia, 1996)
- Determination of Acid Insoluble Ash (Indian Pharmacopoeia, 1996)
- Determination of Water Soluble Ash (Indian Pharmacopoeia, 1996).

**RESULT AND DISCUSSION**

**Pharmacognostical studies**

**Macroscopy**

Thickness of bark varies, usually from 1-1.5 cm according to the age and portion of the plant from where the bark is removed, outer surface, greyish to greyish-brown with ash grey patches, at some places, surface is rough due to a number of lenticels, shallow fissures and a few vertical or longitudinal ridges, inner most surface is smooth and creamy white in colour, the fracture being tough and short, odour, indistinct, taste, slightly bitter.

**Microscopic features**

The bark consist of well defined superficial periderms, narrow cortex, thick major portion of collapsed phloem and narrow zone of non collapsed phloem (Figure- 1) periderms is 150μm thick. It is heterocellular, comprising different cell types. It includes outer portion of phollem and inner derivatives of phelloderm (Figure 2-1). Periderm is a dead tissues, it is heterocellular and includes alternate layers of lignified brachy sclereid and thin walled suberised cells. The suberised cells are called phollemum; the sclereid layers are phelloid(Figure 3-1). Inner to the periderm occurs a thick homocellular zone called phelloderm. The phelloderm cells are thin walled and includes living cells and are storage in function (Figure2,1,3-1). The cortical cells are thin walled and compact. The cells include dense accumulation of circular, concentric starch grains (Figure 3-2). The collapsed phloem forms the widest and more conspicuous zone of the bark. It is heterocellular in organization and includes.

- Highly dilated phloem rays (Figure-1)
- Dilated phloem parenchyma cells
- Crushed and collapsed sieve elements which appears dark, 1 ongential liner found in parenchyma cells (Figure2,2).
- Thick irregular massed of sclereids which are scattered in the outer zone, but occur in discontinuous tangential lines in the inner zone (Figure-1).

**Noncollapsed phloem**

This zone is very narrow and includes intact sieve elements with well preserved companion cells. The cells of the non collapsed phloem are then walled and are organized into regular radial compact lines (Figure 2.2(a). In the noncollapsed phloem region, the phloem rays are narrow and undilated sclerenchyma masses are also lacking.

**Tangential longitudinal sectional view of the phloem(TLS) (Figure-4,1,2)**

As seen in the view, the phloem rays appear non-storied. The rays occur at different levels. The rays are mostly much seriate, short and spindle shaped. Some of the rays are three seriate. All the rays are similar types if polygonal, thin walled and compact. The rays are up to 250 μm in height and 70μm in breadth. The sieve elements are narrow, long and thin walled. The phloem parenchyma cells have several septa and their ends are conical, so that the parenchyma cells appear as fusiform strands (Figure-4,2).

**Radial longitudinal section of the phloem (RLS)**

In RLS view the radial system and vertical system of tissues are visible. The radial system includes phloem rays. The ray appears in horizontal ribbon shaped band of ray elements. The ray cells are homogenous comprising horizontally rectangular or vertical squarish thick walled parenchyma cells. The ray cells do not possess any visible cell inclusions. (Figure- 5.1 and 5.2). The vertical system consists of phloem fibres and phloem parenchyma.
Figure 1: Tangential Sectional View of bark showing periderm, collapsed phloem and non collapsed phloem (TLS).

- CPh-Collapsed phloem,
- DR-Dilated ray,
- NePh-Non collapsed Phloem,
- Pe-Periderm,
- Sc-Sclerenchyma,
- UDR-Undilated ray

Figure 2.1: Tangential Section of periderm portion of the bark (TLS)

- Pm-Phellum, Pe-Periderm, Pd-Phelloderm

Figure 2.2: Tangential Section of Collapsed Phloem enlarged

- CPh-Collapsed phloem,
- PhP-Phloem Parenchyma

Figure 2.2(a): Tangential Section of Non Collapsed Phloem enlarged

- NCPh-Noncollapsed phloem

Figure 3.1: Phellem portion showing pheloids and suberized phellem layers alternating with each other.

- Phd-Phelloderm,
- Ph-Phelloid,
- Pm-Phellem

Figure 3.2: Cortical tissue of the bark possessing dense accumulation of starch grains

- SG-Strach grains
Figure 3.3: A mass of sclereids as seen under polarized light
Σ Sc-Sclereids

Figure 4.1: Tangential Longitudinal Section of bark showing the structure of phloem rays.
Σ Pa- Parenchya
Σ PhR- Phloem Rays

Figure 4.2: Tangential Longitudinal Section of bark showing the structure of phloem rays enlarged(TLS)
Σ PC- Procumbent Cell
Σ PhR- Phloem Ray

Figure 5.1 Radial Longitudinal Section of phloem under low magnification(RLS).
Σ PhS-Phloem Sclerenchyma
Σ PhR- Phloem Ray

Figure 5.2: Radial Longitudinal Section of phloem rays magnified(RLS)
Σ PhR-Phloem Ray

Transverse section of mature stem bark shows, an outer cork composed of thin walled, rectangular and tangentially elongated cells, phellogen single layered, thin walled, tangentially elongated cells followed by a wide secondary cortex, consisting of thin-walled, polygonal to tangentially elongated cells with a number of starch grains, starch grains mostly simple, occasionally compound with 2-3 components also present, large number of stone cells in groups of two or more, found scattered in secondary cortex, single stone cells not very common, stone cells vary in size and shape, being circular to rectangular or elongated with pits and striations on their walls, stone cells distributed somewhat in concentric bands in phloem region except in inner region of phloem which is devoid of stone cells, secondary phloem comparatively a wide zone, consisting of sieve tubes, companion cells, parenchyma and groups of stone cells, alternating with medullary rays, sieve elements found compressed forming ceratenchyma in outer phloem region, whereas in inner region of phloem, intact, medullary rays mostly multiseriate composed of...
thin-walled, radially elongated cells, tangentially elongated towards outer periphery, a number of starch grains similar to secondary cortex also present in phloem and ray cells, few rhomboidal crystals of calcium oxalate also found in this region, inner most layer is cambium.

**Physico-Chemical Parameters**

The physico-Chemical properties such as Ash values, Extractive values and Moisture content of the bark sample are given Table -1.

**TABLE- 1: ASH AND EXTRACTIVE VALUES, LOSS ON DRYING**

<table>
<thead>
<tr>
<th>S.No</th>
<th>PARAMETER</th>
<th>VALUES IN %</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>ASH VALUES</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Total ash</td>
<td>12.45</td>
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<tr>
<td>b</td>
<td>Acid insoluble</td>
<td>0.28</td>
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<tr>
<td>C</td>
<td>Water soluble ash</td>
<td>5.38</td>
</tr>
<tr>
<td>d</td>
<td>Sulphated ash</td>
<td>1.25</td>
</tr>
<tr>
<td>II</td>
<td>EXTRACTIVE VALUES</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Water Soluble</td>
<td>16.5</td>
</tr>
<tr>
<td>b</td>
<td>Alcohol soluble</td>
<td>7.2</td>
</tr>
<tr>
<td>III</td>
<td>Loss on drying</td>
<td>3.8</td>
</tr>
</tbody>
</table>

**TABLE-2: THE CONSISTENCY, COLOUR AND PERCENTAGE YIELD OF THE EXTRACTS OF Crataeva religiosa**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract</th>
<th>Colour</th>
<th>Consistency</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Petroleum ether</td>
<td>Dark green</td>
<td>Semi-solid</td>
<td>3.5</td>
</tr>
<tr>
<td>02</td>
<td>Chloroform</td>
<td>Dark green</td>
<td>Semi-solid</td>
<td>2.7</td>
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<tr>
<td>03</td>
<td>Ethanol</td>
<td>Black</td>
<td>Solid</td>
<td>8.6</td>
</tr>
<tr>
<td>04</td>
<td>Aqueous</td>
<td>Brownish Black</td>
<td>Solid</td>
<td>17</td>
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</tbody>
</table>

**Physico-Chemical Analysis**

**Moisture content**

In the present study the bark sample contains 3.8% moisture. Moisture content (loss of drying) determines the water drying of the drug. Drug containing excess moisture will lead to the activation of enzymes and gives suitable condition for the proliferation of living micro-organisms. Higher water content indicates the presence of larger amount of mucilage or starch and paves way for more chances of microbial degradation and if the value is not too high, it indicates less chance of microbial degradation (Trease and Evans, 1983) Loss on drying is the loss of mass expressed as percent w/w and results are tabulated in Table -1.

**Ash content**

The presence of ash in raw material is determined as total ash, acid insoluble and water soluble ash and sulphated ash. The determination of ash value is useful for detecting exhausted drugs and excess of sandy and earthy matter. The total ash usually consists of carbonates, phosphates and silicates of silica. Ash value determination is a good index of quality and is also helpful to some extent in the detection of adulteration.

An increase in the ash value when compared to the standardized value is indicative of contamination or adulteration (Trease and Evans, 1983).

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs (Mukharjee, 2005). Therefore percentage of the total ash, sulphated ash, acid insoluble ash and water soluble ash were carried out. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction (Khandelwal, 1998).

In the present study, the total ash content of the sample was 12.45%. Acid insoluble ash value can be used to determine the silica impurities admixed with the drug.
while collection and harvesting during rain. Water soluble ash value helps in determining the added mineral matter and helps in the interpolation of analysis of powdered drug for their quality. It is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation (Pruthi, 1980).

Total ash value determined was higher in the mature inner bark than apical and middle bark and increased during summer. Among all the bark samples during both the season, highest amount was noticed in mature inner bark (20.0%) and minimum ash content was estimated for apical stem bark (14.6%). These reports are higher than ash values for *Baccaurea sumatrana* (5.34%) and *Pomelia tomentosa* (1.15%), as reported by Whitten and Whitten, (1987).

The total ash value, water soluble ash value and acid insoluble ash value of *Crataeva tapia* leaf is 12.35%, 0.91% and 5.45% respectively. Since the ash value is constant for the given drugs, this value is one of the diagnostic parameter of the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The aqueous extractive value was found to be higher (27.80%) than the other solvents used viz. benzene, petroleum ether, ethanol and methanol, revealing presence of large amount of water soluble constituents in the leaves. By conventional procedures, loss on drying was performed showing 71.60% loss on drying (Patil et al., 2010).

**Extractive values**

The determination of extractable matter refers to the amount of constituents in a given amount of raw material extracted with suitable solvents. Such extractive values provide an indication of the extraction of the polar and non-polar components present in the material. These values have significance in the evaluation of drugs (Miller, 1973).

Extractive value profiles help in the detection of adulterants during the process of authentication of crude and raw drug materials. Earlier work had revealed the extractive value profiles in several other medicinal plants (Khatoon et al., 2006).

**CONCLUSION**

The present study investigate the authenticity of drug analysed through various pharmacognostical experimental procedure like microscopy, macroscopy, ash values, extractive values and moisture content.

Our research concludes that the pharmacognostical studies support the future identification of *Crataeva religiosa* to experimental purpose.

The future research will be focused on the investigation of bioactivity graded separation and the formulation development may be beneficial to human kind.

**REFERENCE**

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