Pharmacognostic and Physicochemical study of *Punica granatum* L. leaf

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**ABSTRACT**

*Punica granatum* L. (Punicaceae) is a shrub, usually with multiple stems, that commonly grows 1.8-4.6m tall. The deciduous leaves are shiny and about 1-5 cm long. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. Hence, the present work was undertaken to establish the requisite pharmacognostic standards for evaluating the plant material. The present study includes examination of macroscopic and microscopic characters, powder analysis and physiochemical properties of *Punica granatum* L. leaf. The macro and microscopical studies indicated presence of simple leaf, opposite arrangement and prism and cluster crystals of calcium oxalate present throughout the transverse section. Chemomicroscopic characters present included starch and calcium oxalate crystals. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Key words: *Punica granatum* L., chemomicroscopic, pharmacognostic, physicochemical, crystals

**INTRODUCTION**

Plants have been the basis of many traditional medicine systems throughout the world for thousand of years and continue to provide mankind with new remedies. India is represented by rich culture, traditional and natural biodiversity offers a unique opportunity for drug discovery researchers.[1] According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[2,3]

Pharmacognosy has multidisciplinary characters i.e. to identify the drugs, its origin, morphology and microscopic studies, to determine the quality of the drug, its chemical compositions, therapeutic effects, etc.[4] Pharmacognosy studies help in identification and authentication of the plant material. The process of standardization can be achieved by stepwise pharmacognostic studies.[5] The standardization of a crude drug is integral part of establishing its correct identity.

Before any crude drug can be in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established.[6] Therapeutic efficacies of medicinal plants depend upon the quality and quantity of chemical constituents. It has been established that chemical constituents of a plant species vary with regard to climate and seasons.[7]

*Punica granatum* L. (Punicaceae) is a small tree with potential human health benefits, is grown mainly in Iran, India, China, Japan, Russia and USA as well as in most near and far east countries. The pericarp of pomegranate as well as its roots, bark and juice are used in the treatment of colic, colitis-diarrhia, dysentery, leucorrea, hemorrhagia, oxyariasis, paralysis, rectocele and headaches in traditional medicine.[8] The different parts of the tree (leaves, fruits, flowers and bark skin) have been used traditionally for their medicinal properties. The fruit is reported for antioxidant,[9] and cancer prevention.[10] The stem is reported for antibacterial activity.[11] Nair and Chanda,[12,13] reported antichandial activity and antibacterial activity of *P. granatum* leaf. Hepatoprotective role of flowers of *P. granatum* has been reported by Celik et al.[14]

The aim of the present study was to evaluate various pharmacognostic parameters like macroscopic and microscopic characters, powder characteristics and physicochemical properties of *Punica granatum* L. leaf.
MATERIAL AND METHODS

Collection and extraction of plant material
The fresh leaf of *Punica granatum* L. was collected from Jamjodhpur, Gujarat in the month of August 2009. The plant was compared with voucher specimen (voucher specimen No. PSN311) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaf was washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was extracted by sequential method using different solvents. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The acetone extract was used for the solubility study.

Pharmacognostic studies

Macroscopic characteristics
For morphological observations, fresh leaves (approx. 2-3 cm in length) were used. The macromorphological features of the plant parts (leaf) were observed under magnifying lens.

Microscopic characteristics
Free hand section of leaf was taken and stained by well known reagent like safranine to confirm its lignification. Powder microscopy was also carried out and their specific diagnostic characters were drawn and recorded separately.

Physicochemical parameters
Determination of physicochemical parameters as per guidelines of WHO were also performed like total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility, pH analysis, petroleum ether, acetone, methanol and water soluble extractive values, etc.

RESULTS AND DISCUSSION

Macroscopic characteristics
Macroscopically, the leaf was simple in composition, opposite, decussate, oblong-lanceolate or oblong-elliptic, glabrous, subsessile and extipulate. The average leaf size was 2 to 3 cm (length) and 1 cm (width). The fresh leaf was green in color (Figure 1).

Microscopic characteristics
The transverse section of leaf of *Punica granatum* L. showed presence of upper and lower epidermis. The anomocytic and anisocytic stomata were present in epidermis. Unicellular trichomes were present in lamina but less in number. Xylem was lignified, phloem was non lignified, vascular bundles were arc shaped. Prismatic and cluster type of crystals of calcium oxalate were found. Spiral and annular types of xylem vessels were found in transverse section of leaf (Figures 2, 3).

The salient diagnostic characteristics of leaf were arc shaped vascular bundle, anomocytic and anisocytic stomata, reticulate, annular and spiral types of xylem vessels and prism type of calcium oxalate crystal. These characters can be used for standardization of drugs and also used for preparation of plant monographs. Similar study is reported in other plants like *Manilkara hexandra*, *Mitracarpus scaber* Zucc, *Polyalthia longifolia* var. pendula, *Tricosanthes cucumerina* L., *Ricinus communis* L., *Ficus racemosa* Linn., *Anisomeles indica* Linn.
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Diagnostic features of powder were prism and cluster types of crystals of calcium oxalate present on surface of epithelial cells; presence of simple and compound starch grains. In surface view, fragments of epidermis were embedded with anisocytic and anomocytic stomata. Xylem vessels with annular, spiral and reticulated thickening was observed (Figures 4, 5).

Physiochemical properties

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of dry powder of leaves of P. granatum L. was 8.3% which is not very high, hence it would discourage bacteria, fungi or yeast growth. The ash value was determined by three different forms viz., total ash, acid insoluble ash and water soluble ash. The total ash is particularly important in the evaluation of purity of drugs; i.e. the presence or absence of foreign inorganic matter such as metallic salts or silica. Acid insoluble ash measures the amount of silica present, especially as sand. Water soluble ash is the water soluble portion of the total ash. The total ash of crude powder of leaf of P. granatum L. was 7.6%, acid insoluble ash was 6.5% and water soluble ash was 6%. Less amounts of these three parameters show that the inorganic matter and silica was less in leaf of

Figure 3: Microscopic characteristic of Punica granatum L. leaf

Figure 4: Powder study of Punica granatum L. leaf

Figure 5: Powder study of Punica granatum L. leaf
P. granatum L. The extractive value of crude powder was maximum in water (31.09%) and minimum was in petroleum ether (1.46%). pH of acetone extract was 3.45 (Table 1).

Solubility test
The acetone extract of P. granatum L. leaf was evaluated for solubility in 8 solvents with varied polarities. The extract was highly soluble in methanol, dimethylformamide but insoluble in petroleum ether and toluene solvents (Table 2).

CONCLUSIONS
The microscopic characters, the physiochemical studies can be used for the quality control of the crude drug. Such a pharmacognostic study is useful for standardizing crude drugs and can be used to differentiate closely related species. This could also serve in the establishing data for preparation of monograph of this plant. Various physiochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug.

Table 1: Proximate parameters of Punica granatum L. leaf

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value % (w/w)</th>
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<tbody>
<tr>
<td>Loss on drying</td>
<td>8.3%</td>
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<tr>
<td>Total Ash</td>
<td>7.6%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>6.5%</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>6%</td>
</tr>
<tr>
<td>Petroleum ether soluble extractive</td>
<td>1.46%</td>
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<tr>
<td>Acetone soluble extractive</td>
<td>6.23%</td>
</tr>
<tr>
<td>Methanol soluble extractive</td>
<td>27.76%</td>
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<tr>
<td>Water soluble extractive</td>
<td>31.09%</td>
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<tr>
<td>pH</td>
<td>3.45</td>
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Table 2: Solubility of acetone extract of Punica granatum L. leaf

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Solubility (mg/ml)</th>
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<tbody>
<tr>
<td>Petroleum ether</td>
<td>–</td>
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<tr>
<td>Acetone</td>
<td>17</td>
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<tr>
<td>Methanol</td>
<td>232.3</td>
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<td>Ethyl acetate</td>
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<tr>
<td>Toluene</td>
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REFERENCES