



J Ayu Med Sci

Quarterly Journal for Rapid  
Publication  
of Researches  
in Ayurveda  
and Other Traditional  
Medicines

# Journal of Ayurveda Medical Sciences

[www.jayumedsci.com](http://www.jayumedsci.com)

ISSN 2456-4990

## Macro-microscopic characterization of the *Punica granatum* L. flower

*Punica granatum* L. flowers called as Matulampoo in Siddha is a highly medicinal raw material used for the treatment of eye diseases, asthma, diabetes and diarrhoea. A study dealt with the morphology, anatomy and powder microscopy of *P.granatum* dried flowers revealed that the drug consists of bisexual and male flowers. The dried flower is dark red in colour, thick and leathery. Powdered flower drug is creamish white in colour with characteristic smell and taste. The microscopic features recorded from the study will help in authentication of this medicinal material with parameters employed in pharmacopoeial quality control.

Divya et al.



## Macro-microscopic characterization of the *Punica granatum* L. flower

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### ABSTRACT

**Introduction:** Dried flowers of *Punica granatum* L. constitute the drug Matulam poo which is widely used in both traditional and modern medicine. In Siddha it is used for the treatment of diabetes and gastro intestinal diseases. The present study deals with detailed pharmacognosy including morphological, anatomical, quantitative and powder microscopic characterization of this floral drug. **Methods:** Dried authenticated floral drug was collected from SMPG Mettur and subjected to macro-microscopic screening. **Results:** Anatomical study of the sepal showed presence of numerous rosette crystals towards the lower epidermis. Quantitative study revealed the presence of anomocytic stomata in the outer region of sepal. Some of the distinguishing characters observed in the powder microscopy were abundant corolla fragments with anomocytic stomata, epidermal cells with striations, long fibers, calcium oxalate crystals and spherical pollen grains. The current study offers a detailed pharmacognostic profiling of this important floral drug in view of its macro-microscopy.

### KEYWORDS

Matulam, Quantitative microscopy, Rosette crystals

### PICTORIAL ABSTRACT



**ARTICLE HISTORY** Received 26.06.2018 Accepted 17.07.2018

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**CITE THIS RESEARCH AS** Divya KG, Rubeena M, Remya A, Erni B, Brindha S, Sunil Kumar KN, Sathiya Rajeswaran P. Macro – microscopic characterization of the *Punica granatum* flower. J Ayu Med Sci 2018;3(2):384-9.

**DOI** 10.5530/jams.2018.3.12

Indian tradition has immense plant based knowledge on health care. Considered to be symbol of fecundity Pomegranate whose fruit rinds, bark, flowers and roots are used worldwide as taenicides, owing to alkaloids and for the treatment of diarrhea and oral and genital lesions, owing to the presence of tannins and astringency.<sup>[1]</sup> The name pomegranate comes from the Latin Pomum meaning apple and granatus meaning full of seeds. *Punica granatum* L. is known as apple punice from Lythraceae family (previously Punicaceae). Native to central Asia, it is highly adaptive to a wide range of climates and soil conditions and is cultivated along the Mediterranean basin, Asia, and the United States.<sup>[2]</sup>

The tree/fruit consist of several components as: seed, juice, peel, leaf, flower, bark, and roots, each of which exerts intriguing pharmacologic activity.<sup>[3]</sup> *P. granatum* has been used widely in traditional medicine for treatment of diarrhea, dysentery, acidosis, helminthiasis, hemorrhage and urinary

disorders such as kidney stone, bleeding of kidney, irritable condition of bladder inflammation, painful urination, burning sensation, problem in urine discharge.<sup>[4]</sup> *P. granatum* is considered as a diet in convalescence after diarrhea.<sup>[5,6]</sup> It is used in Siddha, Ayurveda and Unani medicine especially for the treatment of gastro-intestinal diseases. The rind of the fruit and flowers, combined with aromatics, such as cloves, cinnamon, coriander, pepper is given as bowel astringent in diarrhea, dysentery and gastralgia.<sup>[7,8]</sup> Pulp is a good anti-diarrhoeal agent.<sup>[9,10]</sup> The extract of *P. granatum* significantly increased the rate of wound contraction and turnover of collagen which is the major component strengthening and supporting the extracellular tissues.<sup>[11]</sup>

In Ayurvedic medicine the pomegranate is considered a pharmacy unto itself and is used as an antiparasitic agent, a blood tonic, and to heal aphthae, diarrhea, and ulcers.<sup>[12]</sup> In Siddha

flower of *P. granatum* is used in the preparation of *Makaelatikulikai* used for the treatment of eye diseases, emesis, diabetes and renal calculus and *Makavacantakucumakaram* used for treating asthma, tuberculosis, inflammation, fever, diarrhoea, stomach pain and anuria.<sup>[13]</sup>

### 1. Plant material

Botanically authenticated dried flowers of *P. granatum* were procured from Siddha Medicinal Plants Garden (SMPG-CCRS), Mettur, Tamil Nadu.

### 2. Macroscopy

Macroscopy was documented by Nikon COOLPIX5400 digital camera. The longitudinal sections of male and hermaphrodite flowers were taken and photographed using Zeiss V8 Stereomicroscope.

### 3. Microscopy

Part of the flower sample was preserved in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml) for

sectioning. Transverse sections of the preserved specimens were hand cut using a 7'o clock platinum blade, stained with safranin and photographed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX5400 digital camera under bright field light. Magnifications were indicated by the scale-bars.

### 4. Quantitative microscopy

Quantitative microscopy of sepal was done boiling about 5 minutes with 10% KOH solution followed by maceration and staining with safranin.

### 5. Powder microscopy

The dried sample was powdered using autoclaved pestle and mortar was powdered, passed through mesh no. 60, and preserved in an air-tight covers for powder microscopy. A pinch of powder was mounted in glycerine on a clean microscopic slide. Slides were observed under Nikon ECLIPSE E200 trinocular microscope and diagnostic characters were identified. Individual characters were magnified to 400X and photographed.

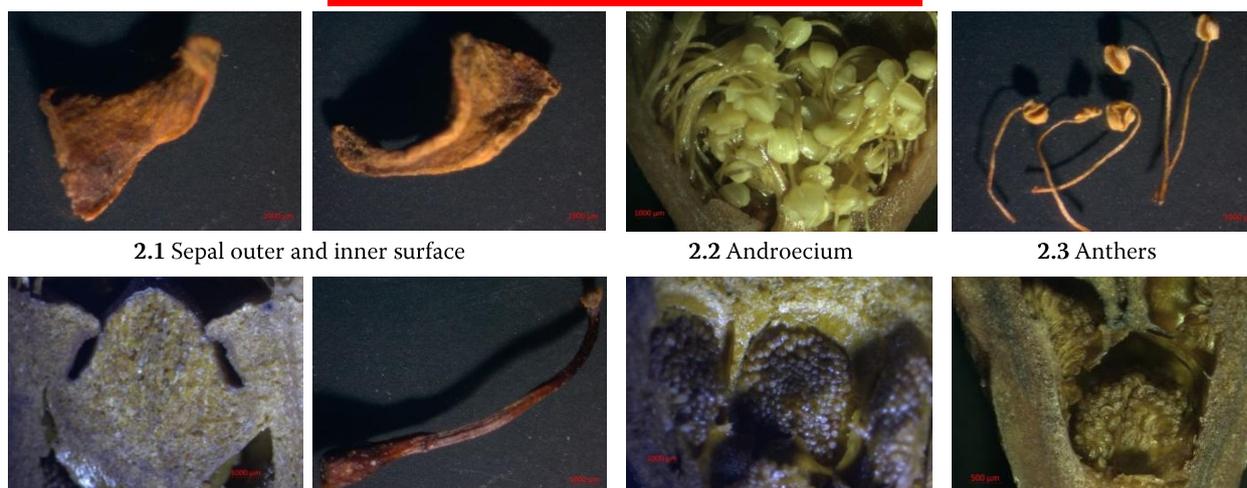
Figure 1. Macroscopy of *Punica granatum* L. flowers



1.1 Heteromorphic flowers - Bisexual and male

1.2 LS of flowers

Figure 2. Individual parts of *Punica granatum* L. flowers



2.1 Sepal outer and inner surface

2.2 Androecium

2.3 Anthers

2.4 Stylopodium

2.6 Style and stigma

2.7 Gynoecium with fertile

ovules

2.8 Rudimentary Ovules

from male flower

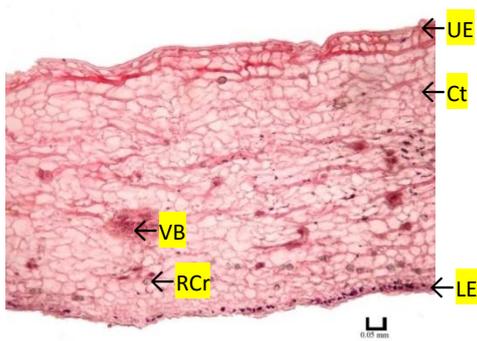
The drug consists of dried bisexual and male flowers of *P. granatum*. Bisexual flowers are vase shaped and have well-formed stigma, style and ovary representing the female parts and filaments and anthers representing the male part; male flowers are

smaller in size and have only well-developed male parts; the bisexual flower measured 2.5 to 4.2 cm l and 0.8 cm to 2.6 cm diameter while the male flowers measures 1.4 to 2 cm in length and 0.6 to 0.8 cm in diameter (Fig 1).

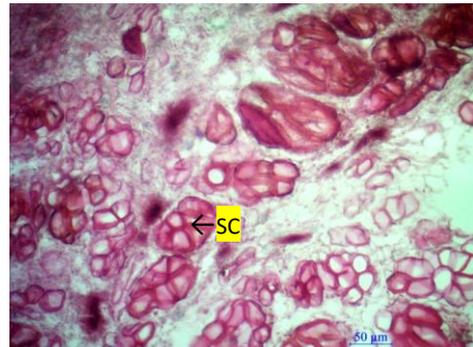
The calyx is red, thick, and leathery fused at the base, and usually pentamerous, although infrequently tetramerous forming the crown of mature fruit; ovary inferior, and the numerous stamens comprising of yellow anthers attached to long red filaments, which are inserted on the inner surface of the calyx tube (Fig 1); anthers surround the elongated style, which at the base broadens into a conical-shaped stylopodium (Fig 2); anthers ditheous and dehisces longitudinally along the median part of the anthers; filament of the stamen is circular in outline; pollen grains spheroidal, tricolpate with a smooth exine and measured about 20 to 21 micrometer in diameter.

The numerous anatropous ovules are seen attached to swollen fleshy parietal placental tissue; the two flower types can be readily distinguished based on differences in pistil development; bisexual flowers possess a well-developed pistil with an elongated style that extends at or above the height of anthers; flowers have a prominent U-shaped ovary containing numerous elliptical ovules. Male flowers have a style that is shorter than the height of anthers and an ovary which is V-shaped; ovules rudimentary, much smaller in size, and have an irregular surface compared with the rounded, cream-colored, and glistening appearance of ovules in bisexual flowers (Fig 2).

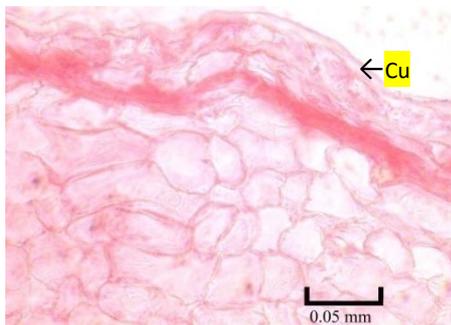
**Figure 3. TS of *Punica granatum* L. sepal**



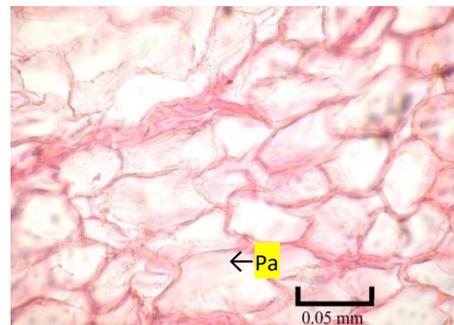
3.1 TS of expanded sepal lobes



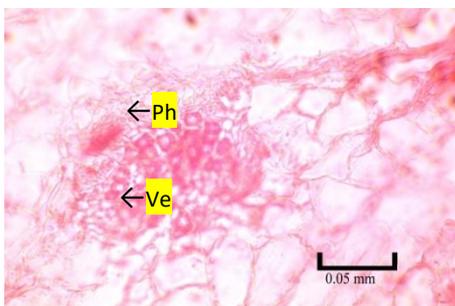
3.2 Outer region of sepal



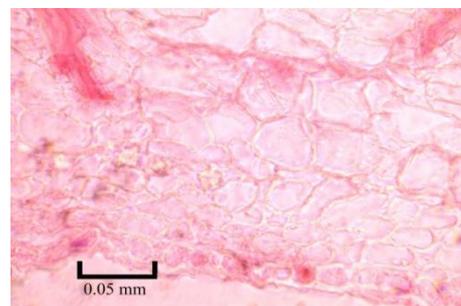
3.3 Upper region



3.4 Middle region



3.5 Vascular region



3.6 Lower region

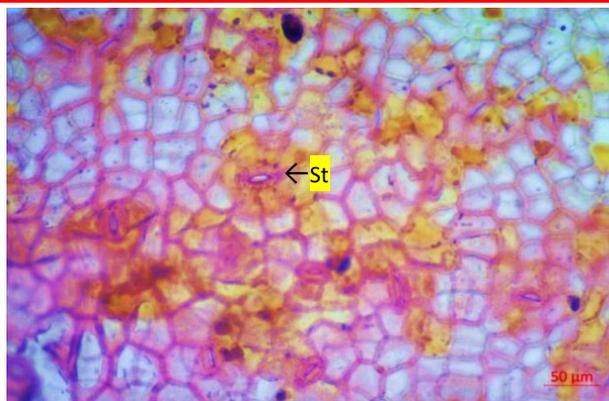
Cu – cuticle; Ct - cortex; LE - lower epidermis; Pa - parenchyma; Ph - phloem; RCr - rosette crystals; SC – stone cells; UE - upper epidermis; VB - vascular bundle; Xy - xylem

Quantitative microscopic features of the sepal has been recorded for authentication of the drug and the results; the epidermal cells of the inner surface showed the presence of unicellular trichomes and were completely absent in the outer

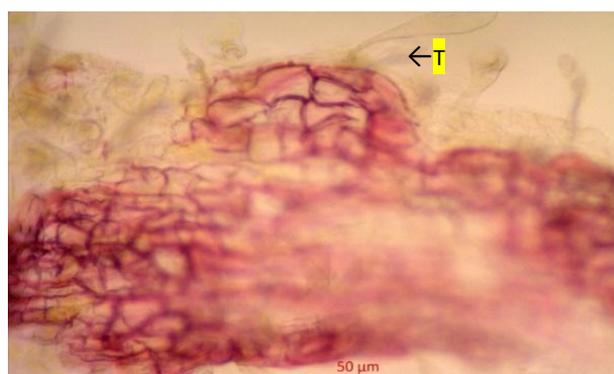
surface the epidermal cell number varied in both the surfaces; anomocytic stomata were present only in outer region of sepal (Table 1 and Fig 4).

Table 1. Quantitative microscopy of *Punica granatum* sepal

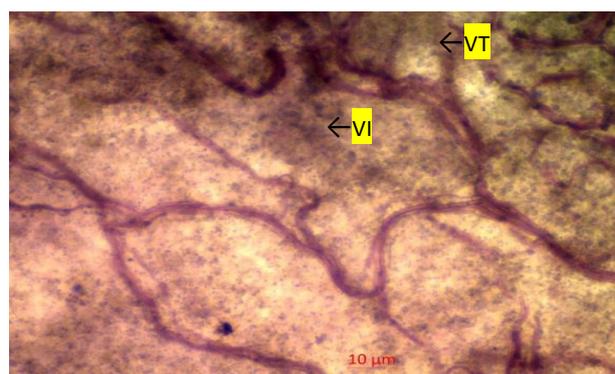
Parameters (/mm <sup>2</sup> )	Outer	Inner
Trichomes	-	54
Epidermal number	220-240	280-320
Stomatal number	10-18	-
Vein termination number		6
Vein-islet number		2

Figure 4. Quantitative microscopy of *Punica granatum* L. sepal

4.1 Epidermal cells with stomata



4.2 Epidermal cells with trichomes



4.3 Vein islets and terminations

St – stomata; T – trichome, VI – vein islet, VT – vein termination

Powdered flower drug showed presence of abundant corolla fragments made of parenchyma cells, thin walled irregularly outlined epidermal cells with striations, trichomes, thick walled parenchyma cells, anomocytic stomata, mesophyll cells of sepal, annular vessels, long fibres, calcium oxalate crystals of both rosette and prismatic type, spherical pollen grains and heteromorphic stone cells (Fig 5).

*Punica granatum* belongs to the monotypic genus which comprises of only two species namely *Punica granatum* and *Protopunica granatum*. Although previously classified under the family Punicaceae recent morphological,<sup>[15]</sup> molecular<sup>[16]</sup> as well as the new classification in the APG IV system<sup>[17]</sup> suggests that it is instead a member of Lythraceae. Chaudhari and Desai classified pomegranate flowers into three types: male, hermaphroditic, and intermediate.<sup>[18]</sup> Observations of gradients of flower types collected during the present study recorded the presence of only

male and hermaphroditic flowers with abundance of male flowers which can be considered as a way to spread genes, because pollen spread is more efficient with more male flowers as recorded by Herlihy and Eckert, 2002<sup>[19]</sup> as well as Tanurdzic and Banks, 2004.<sup>[20]</sup>

Andromonoecy as well as other fluctuating sexual expression types is proposed to allow a species to optimize the allocation of limited resources to male and female function.<sup>[21]</sup> *P. granatum* also showed this condition with the presence of both hermaphroditic and functional male flowers on the same plant.

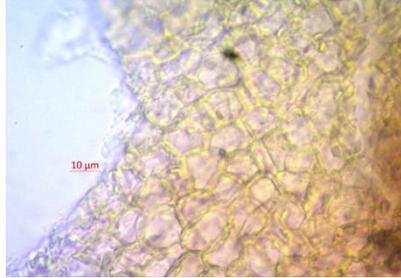
The anatomical studies were in correlation with the studies conducted by Devi et al, 2015<sup>[22]</sup> but differed in the presence of homogenous parenchymatous cortex and comparatively abundance of xylem and phloem. Even though anatomical studies have been carried out earlier this study gives the first hand detailed account of morphology, anatomy,

quantitative and powder microscopic study of the well-known floral drug with immense antiquity.

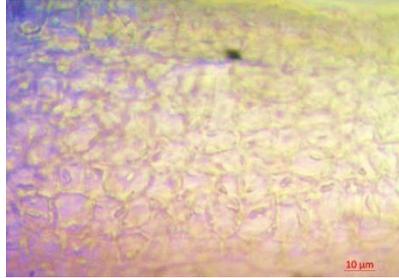
Siddha system of medicine makes use of many flower drugs for their medicinal preparations. Pomegranate flowers have been used in all the three traditional system of medicines viz

Siddha, Unani and Ayurveda as a remedy for various disorders. Thus the present study on the morphology, anatomy and powder microscopy of *P. granatum* will aid in the better understanding of this important floral drug.

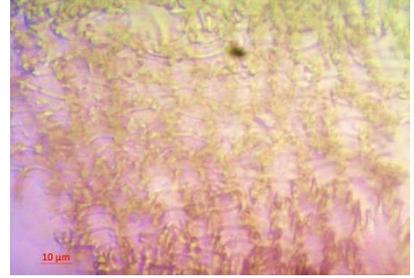
**Figure 5. Powder microscopy of *Punica granatum* L. flower**



5.1 Epidermal cells



5.2 Epidermal cells with anomocytic stomata



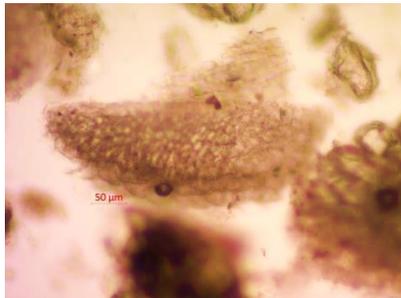
5.3 Epidermal cells with wavy walls



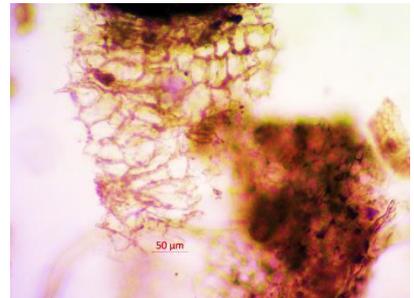
5.4 Trichomes



5.5 Thick walled parenchyma



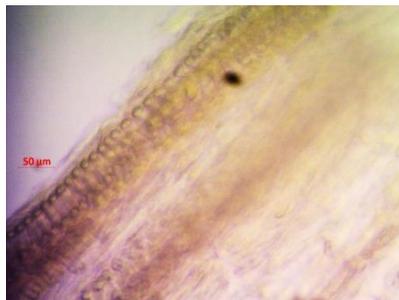
5.6 Mesophyll cells of sepal



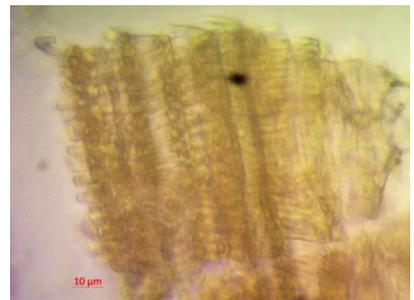
5.7 Thin walled parenchyma



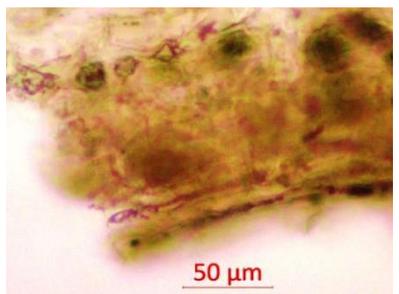
5.8 Fibres



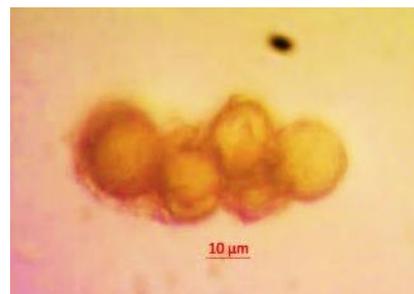
5.9 Vessels



5.10 Stone cells



5.11 Cells with rosette crystals



5.12 Pollen grains

**Acknowledgement** Authors are grateful to Director General, CCRS, Chennai for the support and Dr. M Padma Sorna Subramanian, Research Officer, Siddha Medicinal Plants Garden, Mettur for proving the samples.

**Source of support** Nil

**Conflict of interest** Authors declare no conflict of interest

**Contributors** Dr. KG Divya contributed to the conceptualization of the topic, intellectual content and design, Ms. Rubeena helped in data acquisition and literature study, Ms. Remya Andalil carried out the morphological work, Ms. Erni did the microscopy work, Ms. Brindha carried out the powder microscopy. Dr. KN Sunil Kumar edited the manuscript and provided the suitable suggestions, Dr. Sathyarajeshwaran Parameswaran provided the Siddha aspects of the drug.

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