



## MORPHOLOGICAL AND ANATOMICAL STUDIES ON ORNAMENTAL FLOWERS OF *PUNICA GRANATUM* LINN.

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### ABSTRACT

*Punica granatum* Linn. is a shrub belongs to the family Punicaceae. It is often a cross pollinated food crop and the seeds germinate well. As a result a large number of diverse seedling forms have evolved in nature in various regions of the world over the years. "Double flower"- *Punica granatum* is an ornamental pomegranate type where the flowers have numerous petals and look like a rose flower. These flowers have numerous pharmacological activities and have been used as one of the ingredients in many formulations of Unani system of Medicine. Though the flower has significant therapeutic use since antiquity many fundamental aspects are lacking including the floral biology. Hence, in the present study, an attempt was made to differentiate and describe the morphology and anatomy of the double flower for better understanding it from the normal flowers of *Punica granatum*. Scanning electron microscopic and light microscopic images of various histological sections of the normal flower as well as the double flower clearly exhibit the morphological features and anatomical structure of the flower.

**Keywords:** *Punica granatum* Linn, Double flowers, Anatomical studies, Scanning Electron Microscopic analysis, Unani System of Medicine.

### INTRODUCTION

In India several plants have been subjected for various studies to retrieve their medicinal value. Among various plants of commercial value, pomegranate (*Punica granatum* Linn.) is known worldwide for its delicious taste and health promoting properties. *P. granatum* is member of the Punicaceae family sharing its botanical family only with *Punica protopunica* Balf. F and the species is found only in Socotra Island of the Yemeni coast.<sup>1</sup> Reviews suggest that the plant is considered to be native of Iran, Afghanistan and Baluchistan found growing wild in the valleys and outer hills of the Himalayas between 900 to 1800 m and cultivated throughout India.<sup>2</sup> *P. granatum* is a shrub that naturally tends to develop multiple trunks and has a bushy appearance. Under natural conditions it grows up to a height of 7 m and when domesticated it attains a height of 5 m. All over the world, a good number of different varieties of *P. granatum* were identified. Existence of over 1000 cultivars of *P. granatum* has been recorded from the middle east extending throughout the Mediterranean, East ward to China, India and on to the American Southwest, California and Mexico.<sup>3</sup> In addition, certain *P. granatum* cultivars from India, Russia, China and Turkmenistan were reported as ornamental variety.<sup>4-6</sup> Among the ornamental variety the "Double flower" type and the "Dwarf or Nana type" gains much significant value.<sup>4</sup> The nana type *P. granatum* plant is a miniature pomegranate like "bonsai". It has small leaves, grows to a height of 50-70 cm and bears small flower and fruits.<sup>7,8</sup> The another type of ornamental variety is "Double flower". As the name implies, the flower *P. granatum* produces double flowers wherein numerous stamens are modified into petals. Some of these cultivars of double flowers are fertile and set edible fruits while others are infertile (Abortive). The cause for infertility is due to the modifications seen in the stamens wherein numerous stamens are converted into unusual number of petals. As a result the flowers are large, attractive, look like rose flowers and are called as Double flowers.<sup>4</sup> In Unani system of medicine these infertile double flowers are widely used as medicines in various forms like decoction, powder, syrup, infusion, nasal drop,

gargle, pessery etc under the name "Gulnar" (Persian word for flowers of anar) in treating wide variety of diseases such as diarrhea, dysentery, peptic ulcer, worm infestation, epistaxis, bronchitis, wound healing, leucorrhoea, passive hemorrhages, uterine and rectal ulcers.<sup>9</sup> Though these double flowers possess significant therapeutic use since antiquity, many fundamental aspects are lacking including the aspects of floral biology. Hence the present study was carried out to describe and explore the morphological view and anatomical aspects of various parts of the flower for better understanding it from the normal flowers of the *P. granatum*. Scanning electron microscopic analysis and light microscopic analysis of different sections of both the normal and double flowers clearly exhibit the morphological view and the anatomical arrangement of both the flowers.

### MATERIALS AND METHODS

#### Collection of study material

Normal flowers and Double flowers of *P. granatum* were collected from Herbal garden of Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India during the month of April to June 2012 and were authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai, India. A voucher specimen of normal flowers *P. granatum* (No. 00443) and Double flowers of *P. granatum* (No. 00517) were deposited in the Herbarium of Department of Botany, Captain Srinivasa Murthi Drug Research Institute for Ayurveda and Siddha, Chennai, India. (Figure 1 & 2)

#### Anatomical study

Fresh normal and double flowers of *P. granatum* were collected and the required samples of different organs were cut and fixed in FAA (Formalin-5 ml + Acetic acid-5 ml + 70 % Ethyl alcohol-90 ml). After 24 h of fixing, the specimens were dehydrated with graded series of Tertiary-Butyl Alcohol (TBA).<sup>10</sup> Infiltration of the

specimens was carried out by gradual addition of paraffin wax (melting point 58°-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. De-waxing of the sections was done and sections were stained with toluidine blue.<sup>11,12</sup> Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 Microscopic Unit. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are done as per the standard anatomy books.<sup>13,14</sup>

### Scanning Electron Microscopy

Fresh normal and double flowers of *P. granatum* were collected, fixed in 2.5 % glutaraldehyde (0.1 M phosphate buffer, pH 7.4) at 4°C for 2 h.<sup>15</sup> Tissues were then post-fixed in buffered 1-2 % osmium tetroxide for 1-3 h at 4°C to increase electron density. After dehydration in ethanol and critical point drying with CO<sub>2</sub>, specimens were mounted on to aluminum stubs and coated with silver paint and placed in a vacuum evaporator. After processing, the specimens were observed using a scanning electron microscope (JOEL JSM 6360 LA analytical SEM (JEOL, Tokyo, Japan)) at an accelerating voltage of 10 KV; Photomicrographs were taken at varying magnification at Centre for Advanced Studies (CAS) in Botany, University of Madras, Chennai, India.

## RESULTS AND DISCUSSION

### Anatomical sections

#### Sepal (Normal flowers of *P. granatum*)

The sepal is thick in the middle and becomes gradually thin along the margins. The mid part of the sepal is 1 mm thick. The sepal consists of a thin but distinct epidermal layer of small cylindrical or squarish cells. The ground tissue is homogeneous and parenchymatous. The cells are variable in shape and size and are less compact. Vascular strands are diffusely distributed in the ground tissue and are of different sizes. Some of the strands are small comprising a few xylem and phloem elements which are less prominent. Some are fairly large with a circle of radial lines of small thick walled xylem elements with small clusters of phloem elements. (Figure 3)

#### Sepal (Double flowers of *P. granatum*)

The outermost whorls of perianth are the sepals which are very thick and fleshy. It is 3 mm thick. It is profusely vascularized and parenchymatous. The sepal consists of thick unistratose outer epidermal layer of squarish thin walled cells with thick cuticle. The ground tissue includes scattered clusters of sclereids and thin walled compact angular parenchyma cells. The sclereids are brachysclereid type with thick walls and wide lumen. They are found in groups of two to six cells. In the outer part of the sepal there are small vascular strands which consist of small clusters of xylem and phloem. Towards the inner zone of the sepal the ground parenchyma cells are larger and the sclereids are totally lacking. The vascular strands are larger and variable in structure. Some of the bundles are circular with radiating lines of xylem elements with phloem situated at the outer ends of each xylem strand. There are also somewhat collateral vascular strands which are fan shaped with fairly long chains of xylem elements with phloem elements placed along the outer periphery of the xylem strand. In the ground parenchyma cells of the sepal there are sparsely distributed calcium oxalate druses. (Figure 4)

#### Petal (Normal flowers of *P. granatum*)

The petal is thick in the middle and gradually tapers towards the margin. The midrib region is 600 µm thick. The midrib consists of

thin, rectangular adaxial epidermal cells and fairly thick radially oblong abaxial epidermis with prominent cuticle. The ground tissue is homogeneous and parenchymatous. The ground cells are polyhedral, thin walled and less compact. The vascular strand of the midrib is planoconvex and much extended on the lateral sides. The strand is collateral with several short parallel lines of small xylem element and small clusters of phloem on the abaxial part of the xylem strand. The marginal part of the petal is much reduced in thickness and it consists of central part of 2 or 3 mesophyll tissue and prominent adaxial and abaxial epidermal cells. The epidermal cells are prominently conical. The extreme margin of the petal becomes 2 cells thick. (Figure 5)

#### Petals (Double flowers of *P. granatum*)

The outer petals are thick and the inner petals are comparatively thick. The thin petal has planoconvex midrib with gradually tapering lateral wings. The midrib part is 200 µm thick. It consists of elliptical thin walled adaxial epidermal cells and squarish smaller abaxial epidermal cells. The ground tissue includes angular, small and larger compact stretched vascular strand which includes a few xylem elements and phloem elements. The marginal part of the petal is conical and straight. It is 40 µm thick. It consists of prominent, spindle shaped epidermal cells and horizontal row of wide, rectangular air chamber separated by vertical partition segments. Small vascular strands are located within the partition segments. The thick petals occur at the peripheral part of the flower. It consists of thick and prominent midrib which is 500 µm thick. The epidermal layers of the midrib are prominent. The cells are rectangular and homogenous angular and compact. The vascular strands of the midrib include 3 independent units of xylem elements which are associated with phloem elements. The wings of the thick petal are 120 µm thick. It has quiet large, rectangular epidermal cells with thick cuticle. Some of the epidermal cells are conical in shape. The ground tissue consists of about 4 layers of large, thin walled angular compact parenchyma cells. There are horizontal row of small vascular bundles situated in the median part of the petal. The vascular bundles have thick segments of xylem elements and thin layer of phloem elements both being collateral in position. (Figure 6)

#### Male reproductive parts (Normal flowers of *P. granatum*)

##### Anther

The anther is dithecous and 2 chambered; the anther wall consists of thin epidermal layer and inner endothelial layer which has rectangular with annular thickenings. The anther chamber dehisces longitudinally along the median part of the anther. The filament of the stamen is circular measuring 300 µm in diameter. The filament consists of thick epidermal layer of radially oblong thick walled cells. The ground tissue is parenchymatous thin walled and compact. The vascular strand occurs in the centre of the filament and consists of three short radial lines of xylem alternating with phloem elements. (Figure 7)

##### Pollen grains

The pollen grains are circular with smooth exine. The pollen is 15 µm in diameter. (Figure 8)

#### Male reproductive part (Double flowers of *P. granatum*)

Modifications are seen in the male reproductive parts where the indefinite number of stamens is completely modified into petals.

#### Female reproductive part (Normal flowers of *P. granatum*)

##### Ovary

The ovules are attached on swollen, fleshy parietal placental tissue. The ovules are many and they are anatropous. (Figure 9)

**Ovary (Double flowers of *P. granatum*)**

The ovary exhibits several carpellary chambers and many ovules on parietal placentation. The ovules are anatropous with intact embryo sac. Within the ovule is seen embryo sac with aborted or undeveloped embryo. (Figure 10)

**Pericarp (Normal flowers of *P. granatum*)**

The pericarp is thick and fleshy and fruit a berry. The epicarp of the fruit consists of a thin layer of epidermal cells with thick cuticle. The cells are radially rectangular. The mesocarp is parenchymatous with several scattered vascular strands. The cells of the mesocarp are circular or angular thin walled and compact. The vascular strands are diffuse in distribution. There are larger bundles and small scattered vascular strands. The larger vascular strands are collateral and consist of several short parallel lines of thick walled, angular narrow xylem elements and several discrete phloem strands located on the outer border of the xylem strand. In the inner part of the Pericarp the vascular bundles become small and scattered. They also have collateral xylem and phloem elements. (Figure 11)

**Pericarp (Double flowers of *P. granatum*)**

The pericarp of the fruit is very thick and stony. It includes parenchymatous ground tissue, scattered numerous vascular strands and several masses of brachysclereids. The epidermal layer of the pericarp includes small, squarish thin walled cells followed by 2 layers of similar cells in the sub epidermal region. The ground tissue includes small circular compact parenchyma cells. The sclereids are distributed in outer and inner zones. The middle zone is occupied by

vascular strands. These sclereids are in large discrete masses. The cells are thick walled and lignified. The lumen is wide. The vascular strands are either circular and small or wide and fan shaped. The circular vascular strands are collateral with inner mass of radiating xylem elements with peripheral zone of phloem elements. The fan shaped vascular strands have several radial chains of thick walled angular xylem elements and each line ending in small cluster of phloem. (Figure 12)

**Morphological view of *P. granatum* normal flowers through SEM**

The images obtained through SEM analysis of normal flowers of *P. granatum* are depicted. The normal flowers are shortly pedicelled, have indefinite number of stamens and inserted in the mouth of the calyx. The ovary is inferior with a long style and posses capitates stigma. (Figure 13)

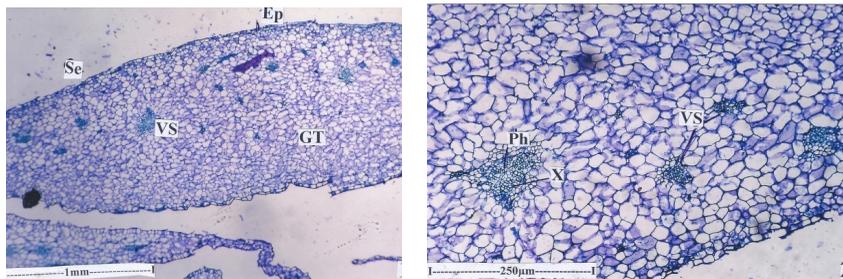
**Morphological view of *P. granatum* double flowers through SEM**

The images obtained through SEM analysis of various parts of ornamental infertile flowers of *P. granatum* are depicted. The calyx is bell shaped with shallow thalamus and encloses a dense crumpled mass of petals. The petals are the actual modification of stamens. The petals in the outermost whorl are longer, but getting smaller towards the periphery. The ovary exhibits several carpellary chambers. Within the ovule many aborted or undeveloped embryo is seen. (Figure 14)



Figure 1: Double flowers of *P. granatum*

Figure 2: Normal flowers of *P. granatum*

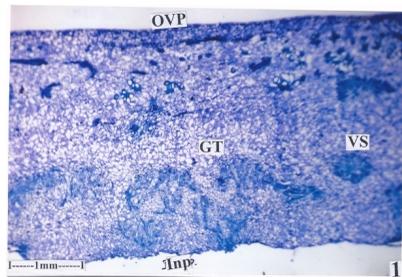


T.S. of sepal entire view

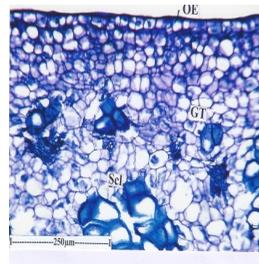
Vascular strands of the sepal – enlarged

EP – Epidermis; GT – Ground Tissue; Ph – Phloem; VS – Vascular Strands; X – Xylem

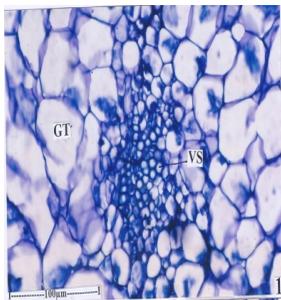
Figure 3: Sepal Portion - normal flowers of *P. granatum*



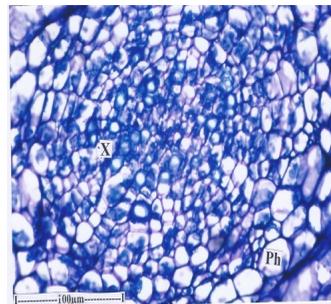
T.S. of sepal entire view



T.S. of sepal outer view showing sclereids masses

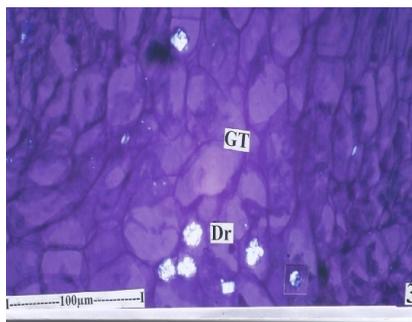


T.S. of sepal with circular vascular strand



T.S. of sepal with small fan shaped vascular strand

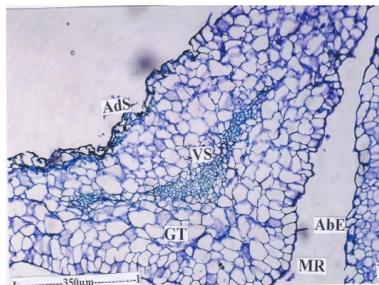
Ep – Epidermis; GT – Ground Tissue; Inp – Inner Part; OE – Outer Epidermis; OVP – Outer Part; Ph – Phloem; Scl – Sclereids; VS – Vascular Strands; X – Xylem



Calcium oxalate druses in the ground cells of the sepal

Dr – Druses; GT – Ground Tissue

Figure 4: Sepal portion - double flowers of *P. granatum*



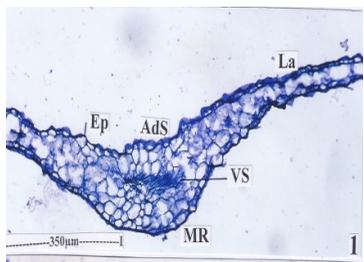
T.S. of thin petal through midrib



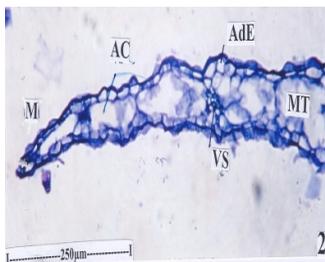
Marginal part of the petal

Abe - Abaxial Epidermis; Ads – Adaxial Side; Ep – Epidermis; GT – Ground Tissue; LW – Lateral Wing; M- Margin; Mr- Midrib; VS – Vascular Strands

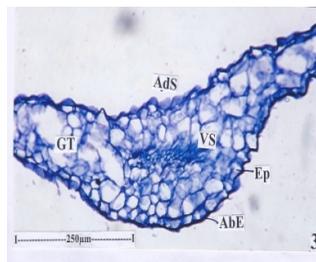
Figure 5: Petal portion – normal flowers of *P. granatum*



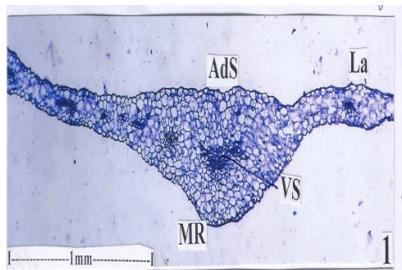
T.S. of thin petal through midrib



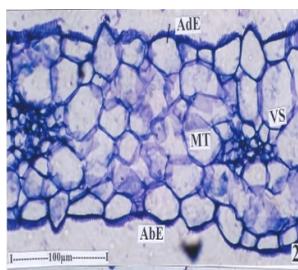
Margin part of thin petal



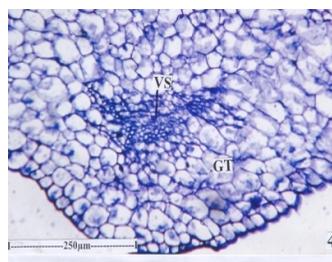
Vascular strand of the midrib of the thin petal



Vascular strand of the thick petal through midrib



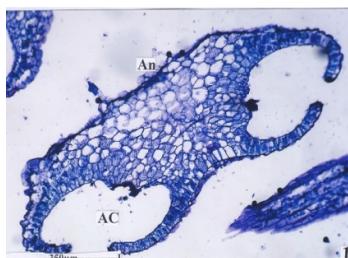
Lamina part of thick petal



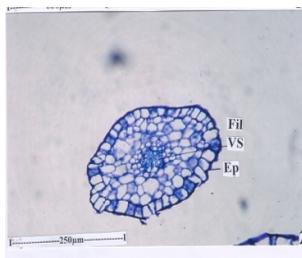
Vascular strand of the midrib of the thick petal

Ads – Adaxial Side; Abe - Abaxial Epidermis; Ac – Air Chamber; Ep – Epidermis; GT – Ground Tissue; La – Lamina; M- Margin; Mr- Midrib; MT – Mesophyll Tissue; VS – Vascular strands

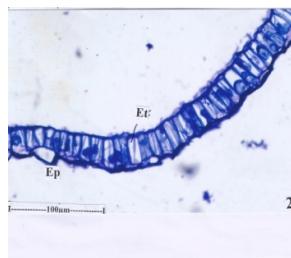
Figure 6: Petal portion - double flowers of *P. granatum*



T.S. of anther



T.S. of the stamen - filament



Anther wall with endothelial cells

AC – Anther Chamber; An – Anther; Fil – Filament; EP- Epidermis; EC – Endothecium; VS – Vascular Strand

Figure 7: Male reproductive parts - normal flowers of *P. granatum*

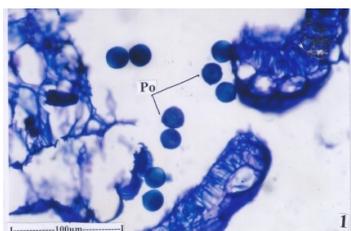
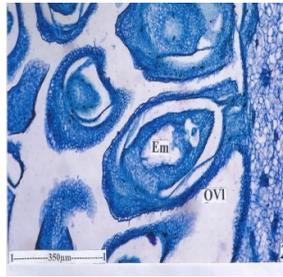


Figure 8: Pollen grains enlarged

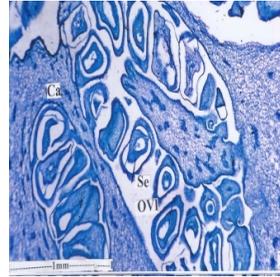


Ovules attached on the swollen superficial placental tissue  
GT – Ground Tissue; Ov – Ovules; PI – Placenta; VS – Vascular strand

Figure 9: Female reproductive part - normal flowers of *P. granatum*



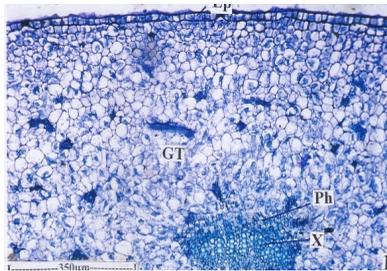
Anatrophous ovule enlarged



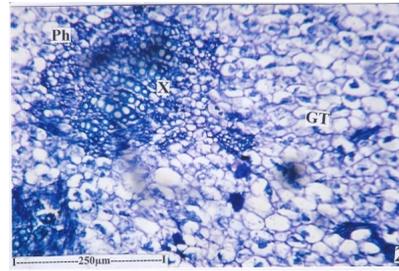
T.S. of ovary showing ovules on placental tissue

Ca – Carpel; Em – Embryo; Ovl – Ovule; Se- Seed

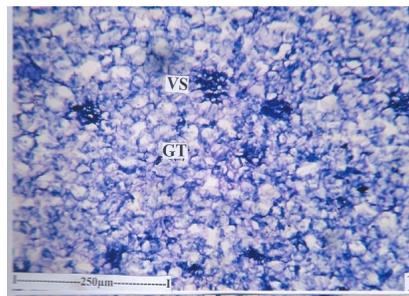
Figure 10: Female reproductive part – double flowers of *P. granatum*



T.S. of pericarp



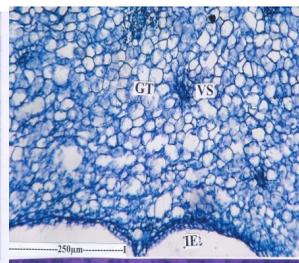
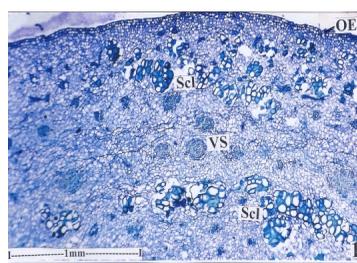
Vascular strands of pericarp



Inner part of pericarp with small vascular strands

GT – Ground Tissue; Phl – Phloem; VS – Vascular Strands; X – Xylem

Figure 11: Pericarp - normal flowers of *P. granatum*



T.S. of pericarp of the outer lamina part of thick petal

GT – Ground Tissue; IE – Inner Epidermis; OE – Outer Epidermis; Phl – Phloem; Scl – Sclereids; VS – Vascular Strands; X – Xylem

Figure 12: Pericarp – double flowers of *P. granatum*

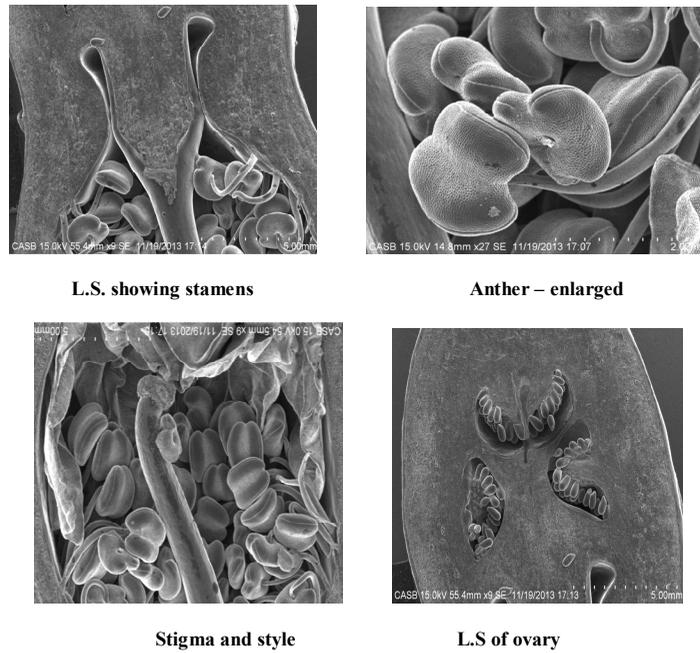


Figure 13: SEM images of *P. granatum* Normal flowers

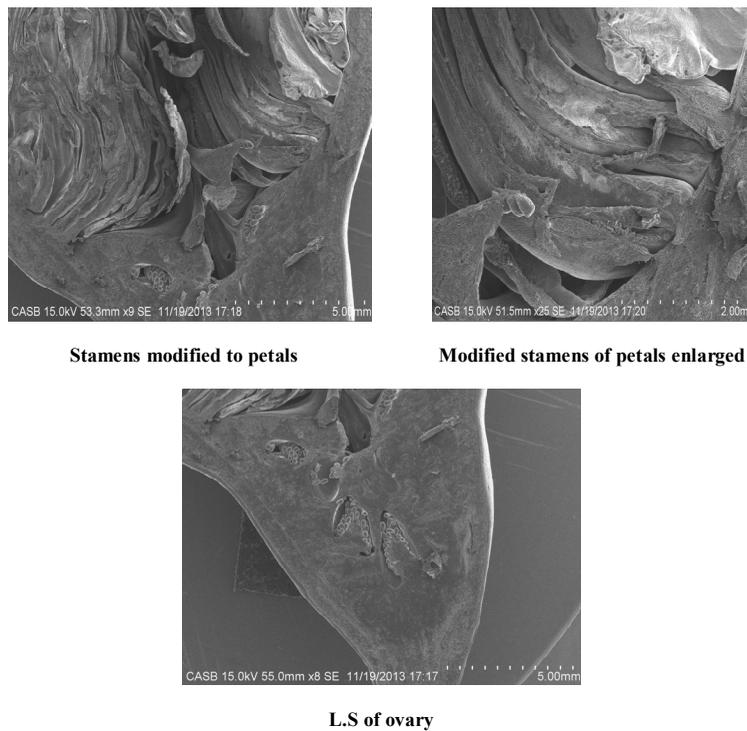


Figure 14: Double flowers of *P. granatum*

## CONCLUSION

As per Unani literature, the double flowers of *P. granatum* claim many therapeutic uses. However more conclusive studies are needed to confirm these effects because there are very few references present in the scientific literature to substantiate these findings. The present research findings on the morphological and anatomical characteristics aided in better understanding the similarity and differences between normal and double flowers of *P. granatum*

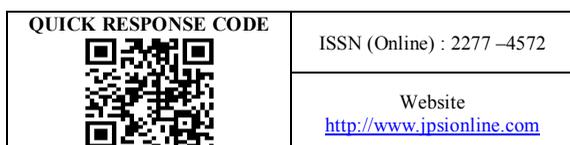
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