

Microscopical Investigation of *Punica granatum* L. Flower: A Traditional Drug with Vivid Therapeutic Promise

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ABSTRACT

Background: The present study was conducted to lay down the microscopic examination of *Punica granatum* L. flower. This taxon's leaves, flowers, fruits and bark are known to possess medicinal properties. It is rich in vitamins C and K, potassium and dietary fiber and has antioxidants, anti-inflammatory, anti-cancer (Prostate), antidiabetic, anti-lipidemic, anti-hypertensive, heart-protective effects, Hepatoprotective and Immune system support properties. **Results:** The microscopy of the flower shows characteristic wing-like structures of the pedicel and its bicollateral vascular bundles, 5 locular ovary; each with more than 2 anatropous ovules, groups of sclereids forming the inner and outer bands in the receptacle that support ovary. Diagnostic features of powder microscopy include tricolpate pollen grains of 15.34 µm to 23.68 µm, tannin-filled cells, pitted sclereids of receptacle measuring 67.66 µm to 113.73 µm, rosette (17.51 µm-24.26 µm), prismatic (22.24 µm-103.52 µm) and rhomboidal (18.95 µm) crystal of calcium oxalate, presence of duct and glandular trichomes. **Conclusion:** The current microscopic study aids not only in the identification of crude drug material but also in establishing the standardization parameters. Further, the results of this study indicated its peculiar characteristics such as the bicollateral position of vascular cells, the presence of rosette crystals of calcium oxalate, the presence of ducts and glandular trichomes exhibiting the presence of secondary metabolites in the flower.

Keywords: *Punica granatum* Linn., Microscopy, Standardization.

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INTRODUCTION

Punica granatum Linn. is commonly known as pomegranate in English and 'anar' in Hindi/Urdu in Indian sub-continent. It is a large deciduous shrub or small tree, growing to a height of about 5-10 m.^[1,2] The plant is mainly found in Iran, the Himalayas in northern India, China, USA and the Mediterranean region.^[1,3]

The leaves, flowers, fruits and bark of *P. granatum* Linn. have been used traditionally used for their medicinal properties,^[3-5] Its fruit is known for the presence of antioxidants^[6] and potential cancer-preventive effects.^[7] The leaf and stem have been reported to possess antibacterial and anticandidal activity, Anti-inflammatory, anti-cancer (Prostate), antidiabetic, Anti-lipidemic, Anti-hypertensive^[5,8-11] and also protect against UV radiation,^[5] while the flowers are reported to be Hepatoprotective^[12] and antidiabetic. *Punica granatum* Linn. is also used in the treatment of carotid artery stenosis, dental

conditions and bacterial infections.^[13] The fruit of this plant is widely consumed throughout the world. It is an important ingredient of traditional remedies for acidosis, diarrhea, dysentery, helminth infection, microbial infections, hemorrhage and respiratory pathologies.^[14] According to the literature, the seeds of this plant contain the estrogenic compounds including estrone and estradiol.^[14,15] In addition, the juice and pericarp are believed to be beneficial in treating various ailments such as allergic, dermatitis, acne, colic, colitis, diuretic, headache, menorrhagia, oxyuriasis, piles and oral diseases.^[16]

In India, *Punica granatum* Linn. is used in the Traditional Ayurveda and Unani system of medicine for the treatment of various ailments. The flower buds of this plant are astringent and useful in chronic diarrhea and dysentery, especially in children. The flower are styptic for the gums and helpful in treating ulcers, vomiting, hydrocele and ophthalmodynia. Flower juice is used against nasal bleeding, and the powder of flower buds is helpful in treating bronchitis. The fruit pulp and the seed of pomegranate assist in digestion and promote appetite.^[17,18]

Various bioactive compounds like alkaloids, anthocyanidins, tannins, flavonoids, phenolics, pro-anthocyanidins, sterols,



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terpenes, terpenoids, xanthonoids, fatty acids, organic acids, lignans, saccharides and vitamin C have been isolated from pomegranate.^[19]

MATERIALS AND METHODS

Sample collection and authentication

The flowers of *Punica granatum* Linn. were collected from the herbal garden of CARI, Bengaluru and dried under shade. All the morphological data of the plant were generated and confirmed from the Flora British India.^[20] The sample is preserved in Raw Drug Museum of RARI, Jammu bearing accession number RARI-JM-200 (Figures 1 and 2).

Freehand Section cutting

The dried flowers along with the pedicel were soaked in warm water for softening. Free hand sections of flower parts were cut with a sharp razor blade. The sections were then cleared in distilled water and studied under Olympus CX41 trinocular microscope. No stains were used, photographs of magnifications of the figure are represented by the scale-bars.

Preparation of powder

Fine powder was prepared from the sample using blender and then sifted through 60 No. sieve as per the standard procedure^[21] and used for powder microscopy.

Powder analysis

Pharmacognostic study was carried on sifted powder by standard method as cited in Ayurvedic Pharmacopoeia of India (API), Part-I, Vol-I, published by Ministry of AYUSH, Govt. of India. The microscopic features were observed using Olympus CX41 trinocular microscope attached with HP desk top by magnus Magcam DC10 (10MP). Whereas macroscopic features were studied under stereomicroscope Olympus SZ2-ILST connected by Magnus Magcam DC5 (5.1MP). The powder microscopy, observations and photographs of the sample was carried out by standard methods under different magnifications.

RESULTS

Flower description

Flowers are bisexual actinomorphic, terminal and solitary or cymose, generally perigynous, a tubular-urceolate hypanthium present and adnate to the ovary. The calyx lobes are 5-8, vulvate, fleshy. The corolla lobes 5-7, imbricated, emerging from the edge of hypanthium. Stamens are very numerous, inserted on the calyx, emerging in many whorls from hypanthium. Anthers 2 celled, 0.1-0.3 cm long, dorsifixed, dehiscent longitudinally; filaments 0.6-0.9 cm long. Pistil one, ovary inferior, locules and carpels 8-12, axil placentation. Style is long and bent, whereas stigma is capitate (Figures 3 and 4).^[2,22]

MICROSCOPY

Pedicel

T.S. of the Pedicel is somewhat circular with wings-like structures. Detailed T.S. shows outer few layers of thick-walled cells filled with brownish content. Cortex wide, composed of parenchyma cells. Rosette crystals are scattered throughout the cortical region. The pericycle is circular and discontinuous. Medullary rays interrupt the pericyclic layer. The phloem is separated into strands by medullary cells. Medullary rays uniseriate. Intraxylem (Bicollateral) phloem present. rosette crystals present in phloem. Xylem capped by Phloem. The xylem consists of abundant fibers and small vessels. The pith is wide and heterogeneous. Rosette crystals of calcium oxalate are also distributed into pith (Figure 5).

Sepal

T.S. of sepal is linear and enclosed within the outer and inner epidermis. The epidermis is covered with a thick cuticle. The epidermal cells are tangentially elongated. It is followed by mesophyll composed of thin-walled parenchyma cells with intercellular spaces. Vascular bundles are present in the center. Rhomboidal crystals of calcium oxalate are present in the mesophyll (Figure 6).

Petal

The T.S. of the petal is more or less V-shaped with distinct outer and inner epidermis. Both epidermises are covered by a thick cuticle. Lower epidermis papillate. The epidermal cells are radially elongated followed by parenchymatous mesophyll. Mesophyll differentiated into an outer palisade, consisting of two layers of radially elongated cells more prominent on the abaxial side and inner spongy cell layers with intercellular spaces. Vascular bundles are present in the center and smaller bundles on the lateral sides (Figure 7).

Androecium

The T.S. of anther shows outer epidermis enclosing endothecium and two anther lobes divided into four pollen sacs and connected by connective tissue. Each pollen sacs are covered by a thick layer of tapetum. The cells of the epidermis are transversely elongated and the middle layers are polygonal. The cells of connective are parenchymatous and a vascular strand is present in the center (Figure 4).

Gynoecium

T.S. of stigma shows epidermis composed of papillose cells. It is covered by thick cuticle. The epidermis is followed by the parenchymatous cell layer. The upper portion of the style is composed of parenchymatous cells with four distinct vascular bundles adjacent to a central canal. In the middle portion and

stylopodium, parenchymatous cells are confined to outer 3-4 layers and rest of the ground tissue has abundant sclereids.

The TS of the ovary shows epidermal cells of ovary wall which are tangentially elongated and covered by a thick layer of cuticle. The ground tissue is parenchymatous and possesses bands of vascular bundles and groups of sclereids. In the center, the 5 locules are separated by layers of septum. Each locule consists of many anatropous ovules (Figure 8).

Receptacle

The T.S. of the receptacle is circular. The epidermis is covered by a thick cuticle and composed of tangentially elongated cells. It is followed by 5-6 layers of thick-walled cells. The central region consists of oval to oblong cells and bears groups of sclereids arranged in an outer and an inner band. The inner band consists of abundant pitted sclereids (Figure 9).

POWDER MICROSCOPY

Organoleptic study

The powder of flower of *Punica granatum* Linn. appears saddle brown in colour. Odour pungent, taste is slightly bitter and the texture is smooth.

Microscopy

Powder of *Punica granatum* Linn. consists of unicellular and uniseriate trichome fragments; sessile glandular trichomes of around 30.09 μm long, epidermal cells with portion of the cortical

cell, parenchyma cells and fibers; papillae of petal, xylem fibers, pollen grains of 15.34 μm to 23.68 μm in singly and in cluster, tannin filled cells, pitted sclereids of receptacle of 67.66 μm to 113.73 μm in size, spiral and annular thickened xylem vessels; rosette (17.51 μm -24.26 μm), prismatic (22.24 μm -103.52 μm) and rhomboidal (18.95 μm) crystal of calcium oxalate (Figures 10 and 11).

DISCUSSION

Punica granatum (commonly known as pomegranate) is a fruit-bearing plant and its flowers have a notable pharmacognostic profile due to their medicinal properties. Pomegranate flowers contain several bioactive compounds, such as flavonoids, phenolic compounds, alkaloids and tannins, which contribute to their therapeutic effects.^[19] The flowers of *Punica granatum* have been used in traditional medicine systems, especially in Ayurvedic and Unani practices, for various health benefits,^[5,23] Various pharmacognostic studies have been carried out on the peels,^[4,11] leaves,^[3] flower buds,^[2] and seeds,^[24] which also report the presence of tannins, sclereids and prismatic and rhomboidal crystals of calcium oxalate. Like the leaves,^[3,24] the flowers also contain fibers. The collateral vascular bundle is a prominent feature of the pedicel. The epidermal cells of the sepal are tangentially elongated in the mature flower; however, they are radially oblong during the bud stage. The epidermal cells of the petal are papillate and squarish in shape during the bud stage.^[2] The ovary has a thick wall and the epidermal layers consist of tangentially elongated cells, while the epidermal cells of the



Figure 1: Plant Habitat and close view of *Punica granatum* L.



Figure 2: Dried flower of *punica granatum* L.

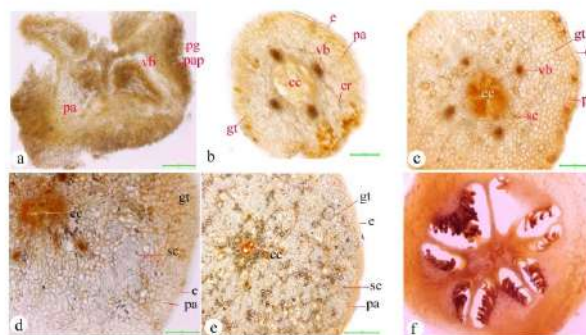
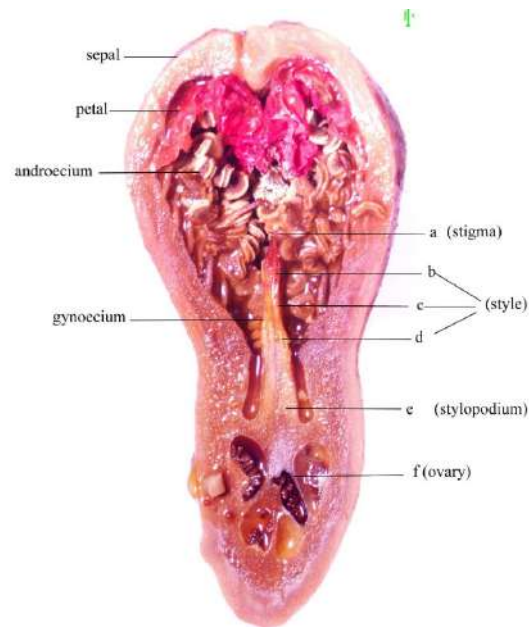


Figure 3: Stereo photo macrograph of the flower bud of *Punica granatum* L. T.S of pistil; stigma (a); style (b-d) stylopodium (e) and ovary (e). Abbreviations: pg, pollen grain; pap, papillae; vb, vascular bundle; pa, parenchyma; e, epidermis; cr, crystal; gt, ground tissue; sc, sclereid; cc, center canal.

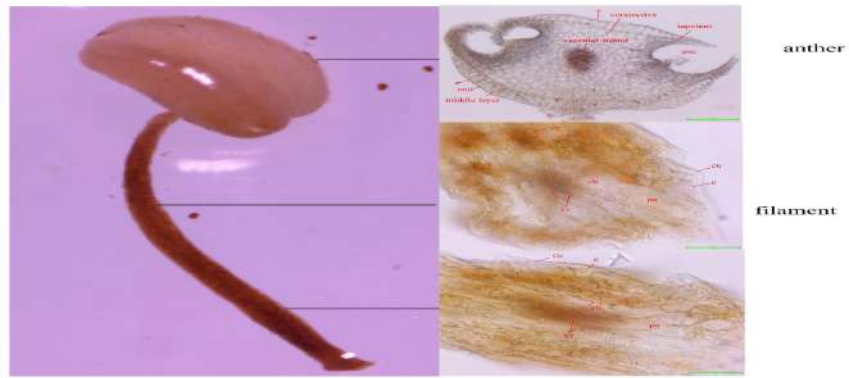


Figure 4: Stereo photomicrograph of androecium of *Punica granatum* Linn. T.S. of anther and filament. Abbreviations: e, epidermis; cu, cuticle; vb, vascular bundle; pa parenchyma; xv, xylem vessel.

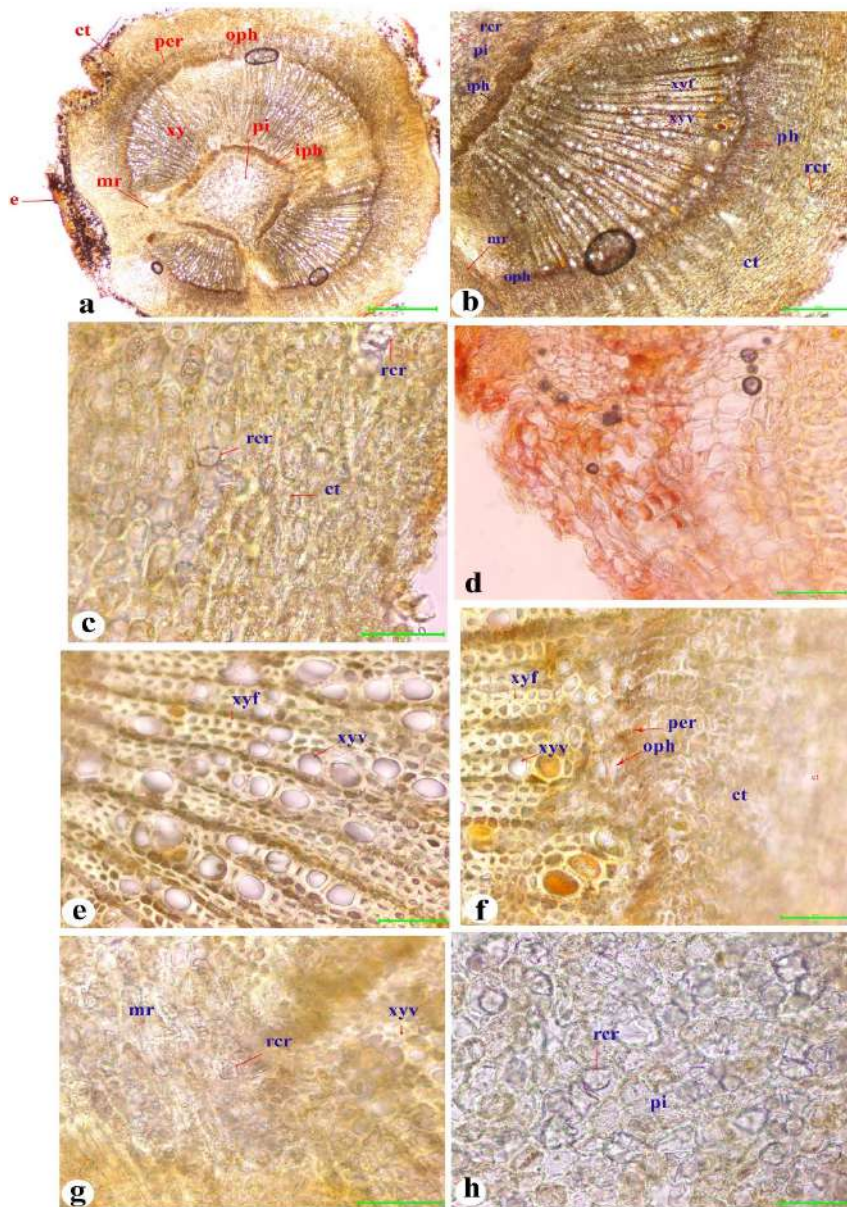


Figure 5: T.S. of Pedicel of the flower of *Punica granatum* Linn. (a-h). Abbreviations: ct, cortex; per, pericycle; oph, outer phloem; xy, xylem; iph, inner phloem; xyv, xylem vessel; xyf, xylem fiber; mr, medullary ray; pi, pith; rcr, rosette crystal of calcium oxalate.

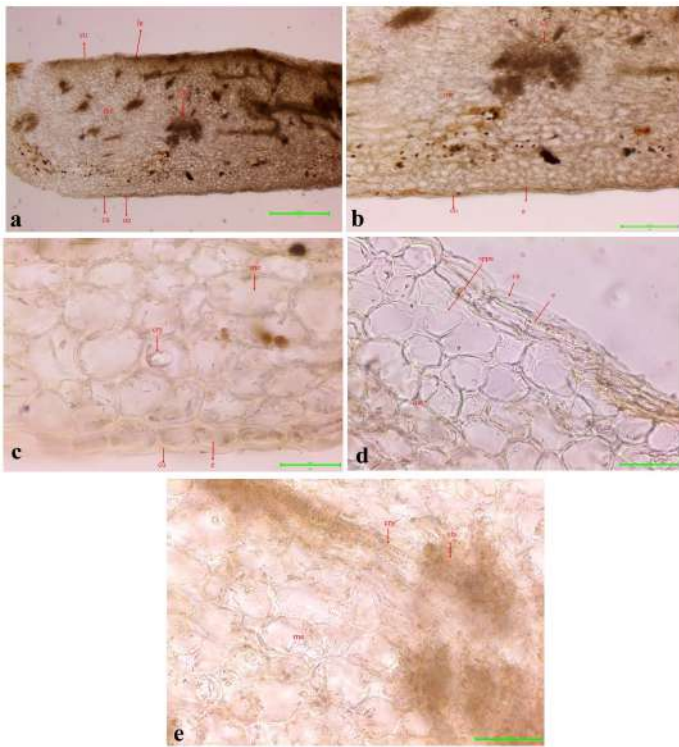


Figure 6: T.S. of Sepal of the flower of *Punica granatum* Linn. (a-e). Abbreviations: cu, cuticle; le, lower epidermis; me, mesophyll; vb, vascular bundle; oe, outer epidermis; cry, crystal of calcium oxalate; sppa, spongy parenchyma.

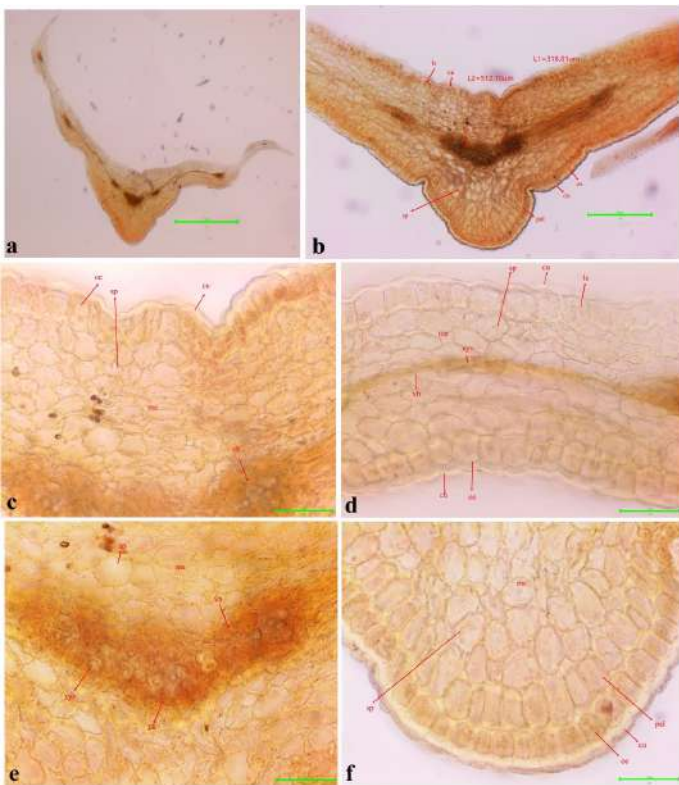


Figure 7: T.S. of Petal of the flower of *Punica granatum* Linn. (a-f). Abbreviations: cu, cuticle; le, lower epidermis; me, mesophyll; vb, vascular bundle; xyv, xylem vessel; oe, outer epidermis; cry, crystal of calcium oxalate; pal, palisade; sp, spongy parenchyma.

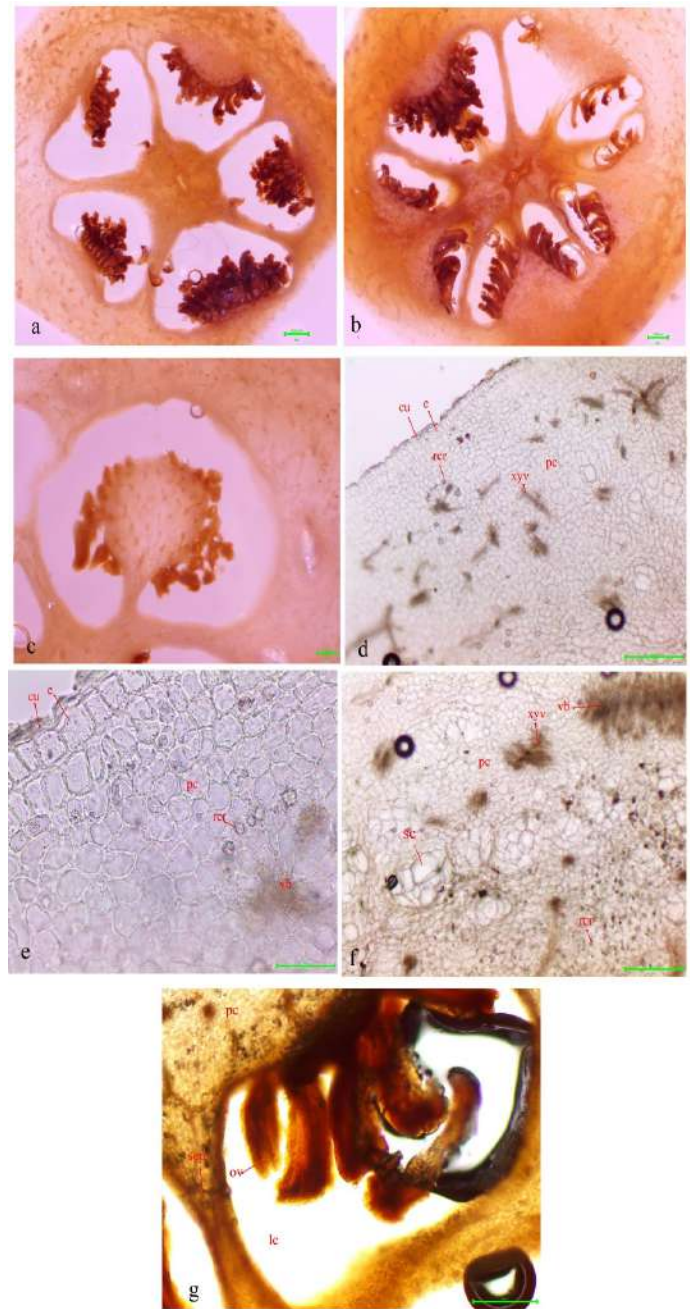


Figure 8: T.S. of Ovary of *Punica granatum* Linn. (a-f). (a-c) under stereomicroscope and (d-f) compound microscope. Abbreviations: cu, cuticle; e, epidermis; xyv, xylem vessel; vb, vascular bundle; rcr, rosette crystal of calcium oxalate; sc, sclereid; pc, parenchyma; sep, septum; lc, locule; ov, ovule.

stigma are papillate. Vascular strands and patches of sclereids are scattered in the parenchymatous ground tissue of the ovary. There are five locules, separated by layers of septum, present in the center. Each locule contains many anatropous ovules. The outer epidermis of the dithecous anther encloses the endothecium and is connected by connective tissue. The microsporangia are covered by a thick layer of tapetum and the vascular strand is present in the center. The central region of the receptacle consists of oval cells and bears groups of sclereids arranged in an outer

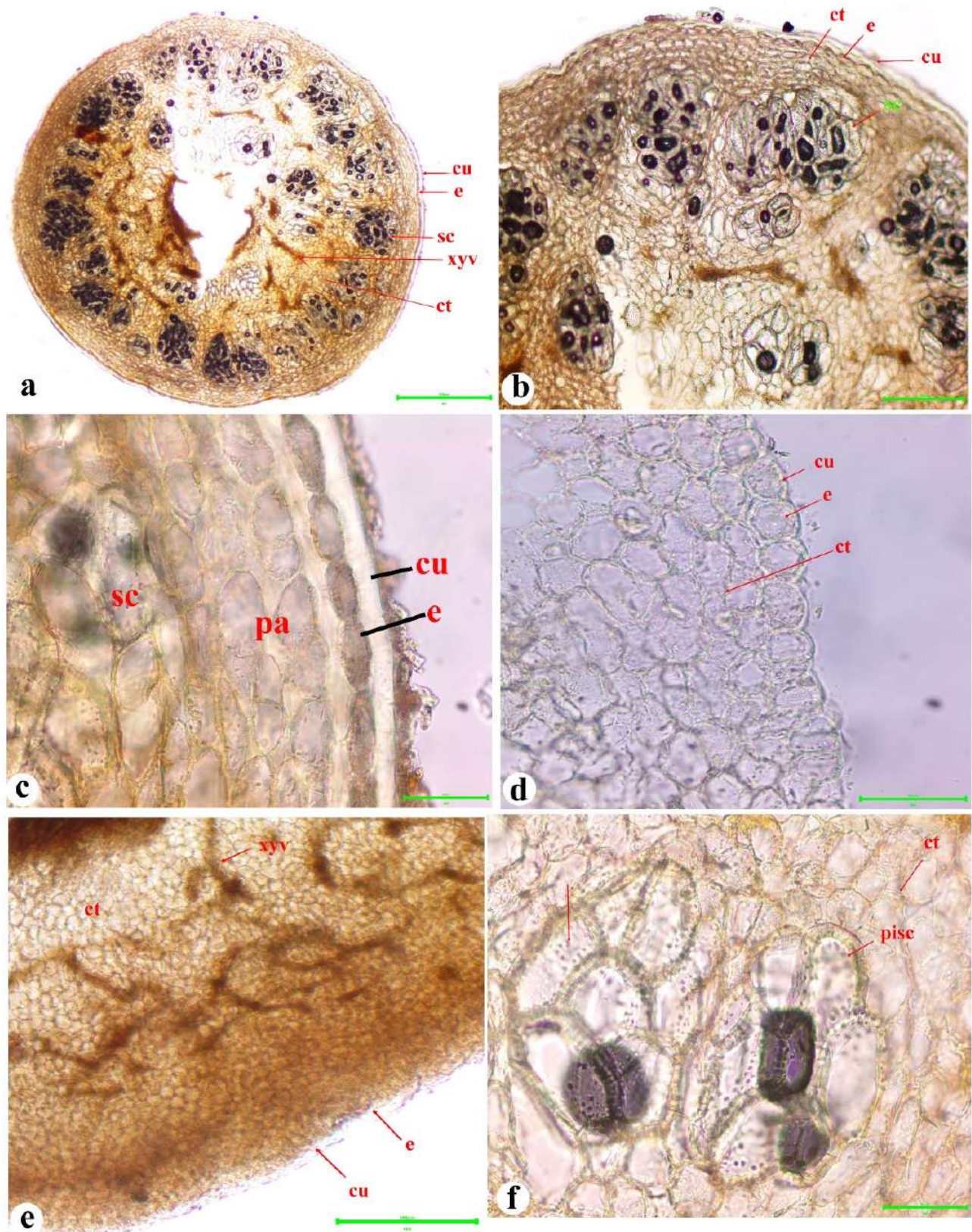


Figure 9: T.S. of the receptacle of the flower of *Punica granatum* Linn. (a-f). Abbreviations: cu, cuticle; e, epidermis; pa, parenchyma; xyv, xylem vessel; sc, sclereid; ct, cortex; pisc, pitted sclereid.

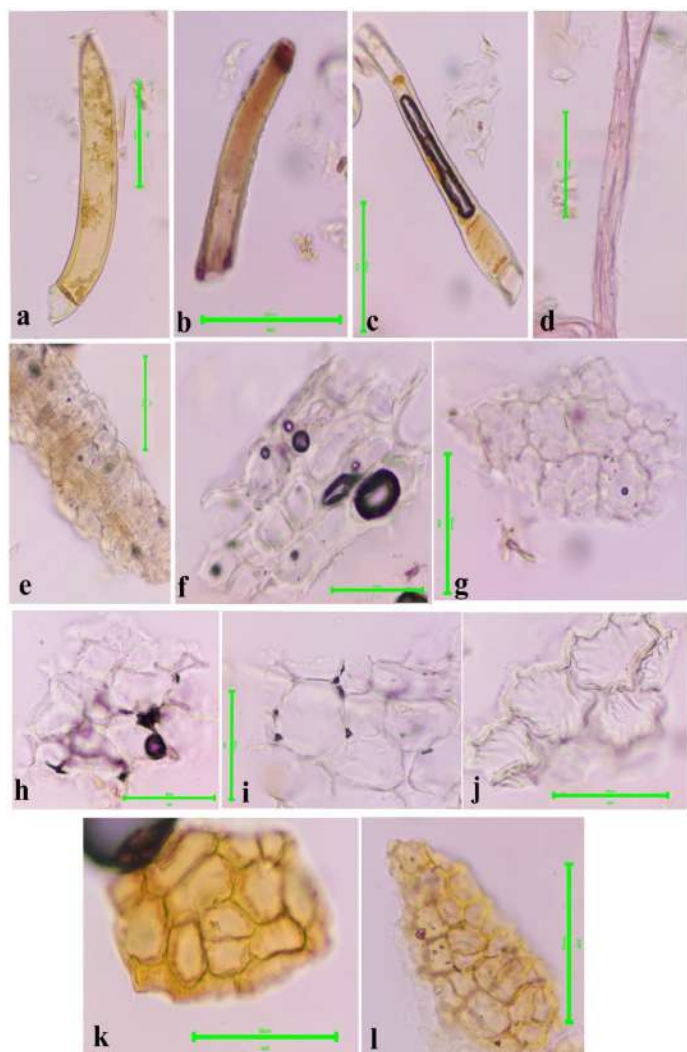


Figure 10: Powder microscopy of the flower of *Punica granatum* Linn. Segment of trichome (a-c); laticifer canal (d); epidermal cell with segment of the cortex (e); Fragment of parenchyma cells (f-l).

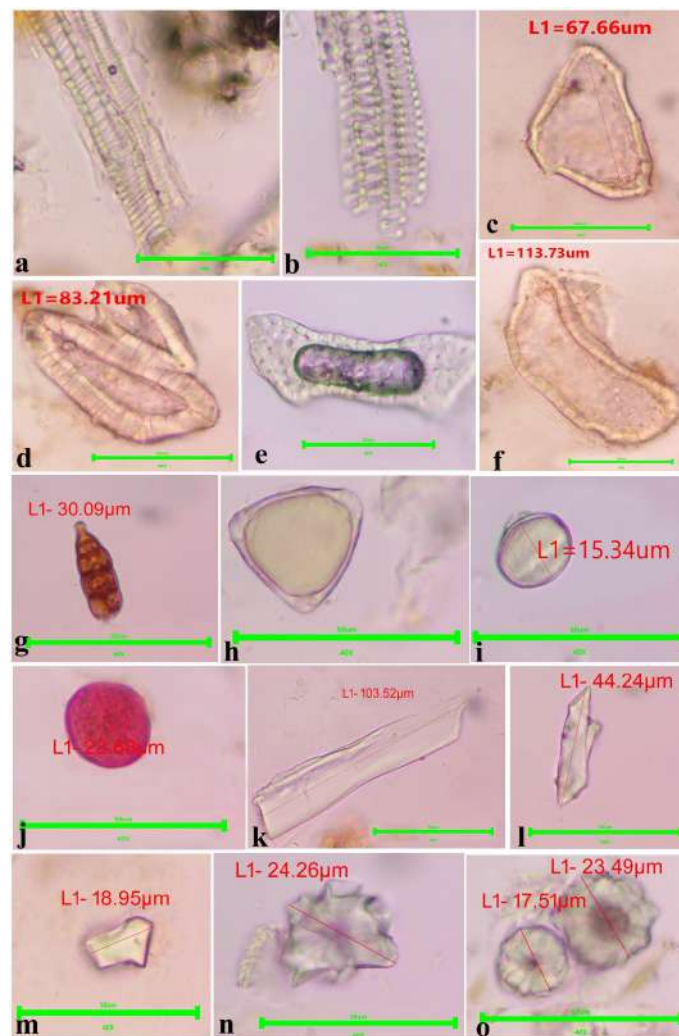


Figure 11: Powder microscopy of the flower of *Punica granatum* Linn. Annular and spiral xylem vessel (a-b); sclereids (c-f); glandular trichome (g); pollen grains (h-j) (h); rosette, prismatic and rhomboidal crystal of calcium oxalate (k-o).

and an inner band. The inner band consists of abundant pitted sclereids that support the ovary from the base.

CONCLUSION

The anatomical study of *Punica granatum* L. reveals several characteristic features that can be easily observed in the powder microscopy microscopy. These include the bicollateral arrangement of vascular bundles and the presence of a large number of sclereids supporting the ovary. Rosette crystals of calcium oxalate are evenly distributed in the cortical region and the pith. The presence of ducts and glandular trichomes in the flowers indicates the presence of secondary metabolites. These characteristic features aid in the identification of the genus and are useful for further investigation of crude drug materials from the species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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ABBREVIATIONS

UV: Ultraviolet, **RARI;** Regional Ayurveda Research Institute, **API:** Ayurvedic Pharmacopoeia of India, **CARI:** Central Ayurveda Research Institute, **CCRAS:** Central Council for Research in Ayurvedic Science, **AYUSH:** Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy

SUMMARY

The study deals with the microscopical investigations of *Punica granatum* L. flower collected from the herbal garden of CARI, Bengaluru. Macro and microscopic studies and powder microscopy were carried out for the identification of crude drug material and to establish the standardization parameters. The results of the study showed its peculiar characteristics such as the bicollateral position of vascular cells, the presence of rosette crystals of calcium oxalate, the presence of ducts and glandular trichomes exhibiting the presence of secondary metabolites in the flowers.

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