

## Pharmacognostic Evaluation of the Rind of Ganesh and Kabul Variety of *Punica granatum* Linn– a Comparative Study

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### ABSTRACT

Pomegranate (*Punica granatum*), a small tree originating in the orient, belongs to the *Punicaceae* family. *P. granatum* is grown mainly in Iran, India and the USA, but also in far East countries. The most important use of *P. granatum* is as table fruit, but large amounts are used in the beverage and liquor industries. The pericarp, containing up to 30% tannins, is used in tanning leather. In Sanskrit it is known as “Dadima”, and Hindi as Anar and in Tamil as Madulai. The rinds of fruits are valued as astringents in diarrhea and dysentery. In folk medicine, dried pericarp of pomegranate preparations and the juice of the fruits are employed as an oral medication in the treatment of colic, colitis, leucorrhea, menorrhagia, oxyuriasis, paralysis and rectocele. This study deals with the pharmacognostic evaluation of the two variety of rind belong to *punicaceae* family which includes microscopic studies like transverse section of the rind. In conclusion in both the transverse section of the rind we observed the presence of epidermis, mesocarp, vascular strand and cuticle are seen.

**Keywords:** *Punica granatum*, *Punicaceae*, pericarp, pharmacognostic, microscopic.

### INTRODUCTION

The pomegranate tree, *Punica granatum*, and especially its fruit, has a vast history of uses for the treatment of medical and health related issues. Pomegranate, the fruit of the *Punica granatum* tree which is a long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. It belongs to the *Punicaceae* family.<sup>[1]</sup> The foremost use of pomegranate is as table fruit, but large amounts are used in the beverage and liquor industries.<sup>[2]</sup> The pericarp, containing up to 30% tannins, is used in tanning leather.<sup>[3]</sup> In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer disease, arthritis, and obesity. Medically beneficial compounds can be derived from seed, juice, rind, leaf, flower, bark, and roots of pomegranate. Each of these anatomical compartments of the plant has interesting pharmacologic activity. The rinds of fruits are valued as astringents in diarrhea and dysentery.<sup>[4-6]</sup> In folk medicine, dried pericarp of pomegranate preparations and juice of fruits

are employed as an oral medication in the treatment of colic, colitis, leucorrhea, menorrhagia, oxyuriasis, paralysis and rectocele and external application to swollen breast<sup>[7]</sup> and the nape of the neck in mumps<sup>[8]</sup> and headache.<sup>[9]</sup> A number of pharmacological effects of these materials have been described including vermifugal, taenicial, astringent, antispasmodic, antihysterical and diuretic.<sup>[10]</sup> Pomegranate peel is used for treating infection of male or female sexual organs, mastitis, acne, folliculitis, piles, allergic dermatitis, tympanitis and for the treatment of oral diseases. The present investigation was planned with an intention to establish and compare the pharmacognostic standards of two variety of the rind of *Punica granatum* Linn that can facilitate the authentication of the correct variety to be used for further study.

### MATERIALS AND METHODS

#### Plant material

Two variety of plant specimen for the proposed study was collected from local fruit market. Care was taken to select healthy fruits. It was identified as *P. granatum* Linn having yellowish red rind (Ganesh variety) and reddish rind (Kabul variety) belongs to *Punicaceae* family. The required fruit rind were cut and removed from the fruit and fixed and cast in to paraffin blocks. It was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Chennai. A voucher specimen is maintained in plant anatomy research centre, Chennai. The fresh rind of the fruit was used for the microscopic studies.

#### Collection of specimen

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The required rind was cut and removed from the fruit and fixed in FAA (formalin 5 ml + acetic acid-5ml + 70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. [11] Infiltration of the specimens were carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation and specimens were cast into paraffin blocks.

### Sectioning

Paraffin embedded specimens were sectioned using Rotary Microtome of thickness 10-12µm. Dewaxing of the sections were carried out by customary procedure. [12] Sections were stained with Toluidine blue, a polychromatic stain as per the method published by O'Brien *et al.* [13] Staining results were remarkably good; and cytochemical reactions were also observed. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies.

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 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 components were studied and measured.

### Photomicrographs

Photographs of different magnification were taken using Nikon lab photo 2 microscopic units; microscopic descriptions of tissues were supplemented with micrographs wherever necessary. 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 were indicated by the scale bars. Descriptive terms of the anatomical features were followed as given in the standard anatomical books. [14]

### RESULT AND DISCUSSION

The detailed pharmacognostic evaluation would give valuable information for further studies.

Fig. 1: Anatomy of the Fruit rind (Ganesh variety)

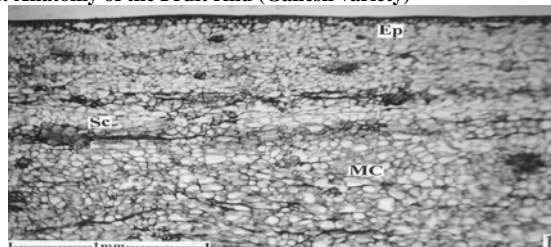


Fig. 1a: T.S of fruit rind – Entire view Ep- Epidermis; MC- Mesocarp; Sc- Sclereids.

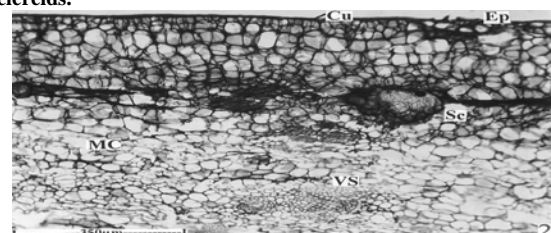


Fig. 1b: Upper portion of the fruit rind magnified. Ep- Epidermis; MC- Mesocarp; Sc- Sclereids; VS- vascular strand; Cu- cuticle.

Fig. 2: Anatomy of the septa (Ganesh variety)

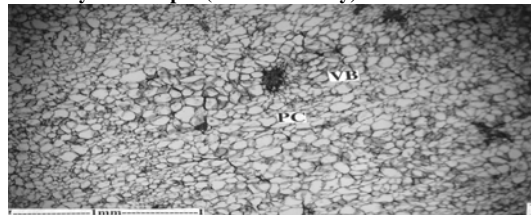


Fig. 2a: T.S of outer septa with vascular bundles VB- Vascular bundle; PC- Parenchyma cell

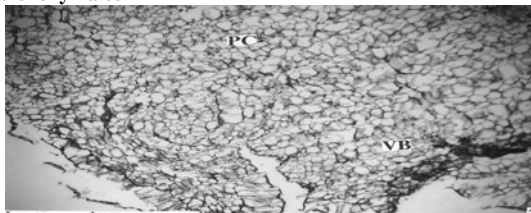


Fig. 2b: T.S of inner septa VB- Vascular bundle; PC- Parenchyma cell

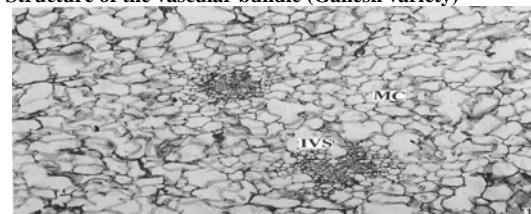


Fig. 3a: T.S of rind showing inner vascular bundle MC- Mesocarp; IVS- Inner vascular bundle

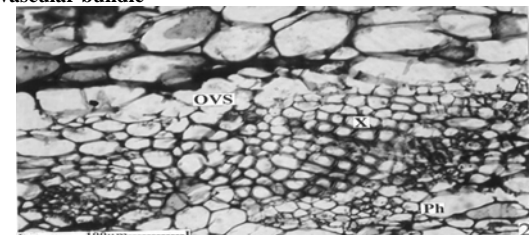


Fig. 3b: T.S of rind showing outer vascular bundle OVS- Outer vascular bundle; Ph- phloem; X- Xylem

Fig. 4: Distribution of the sclereids and starch grains (Ganesh Variety)

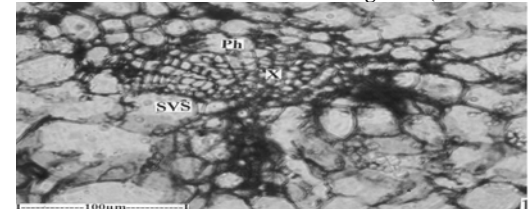


Fig. 4a: T.S of septa through vascular bundles Ph- phloem; X-Xylem; SVS- Septal vascular strand

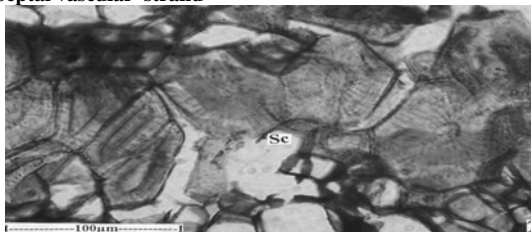


Fig. 4b: T.S of outer rind showing sclereids distribution Sc- Sclereids

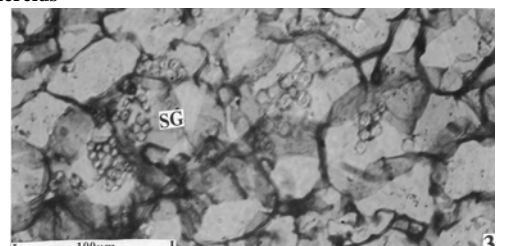


Fig. 4c: starch grains in the septal region SG- Starch grains

Fig. 5: Anatomy of the fruit rind (kabul variety)

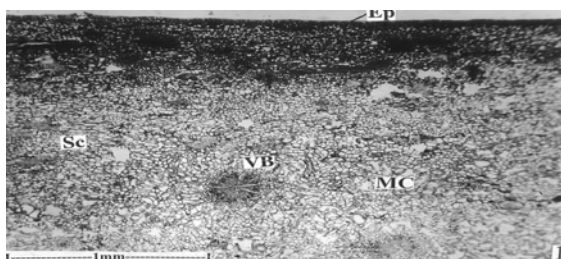


Fig. 5a: T.S of fruit rind – Entire view

Ep-Epidermis; MC- Mesocarp; Sc- Sclereids;VS- Vascular bundle

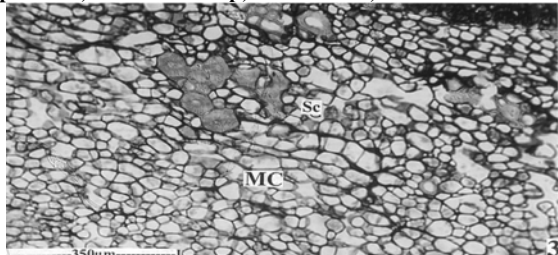


Fig. 5b: T.S of the fruit rind – outer sclerotic region magnified  
MC- Mesocarp; Sc- Sclereids;

Fig. 6: Structure of the vascular bundle (kabul variety)

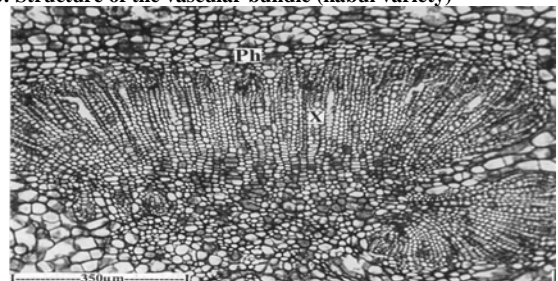


Fig. 6a: T.S of rind showing outer vascular bundle magnified  
Ph- phloem; X-Xylem

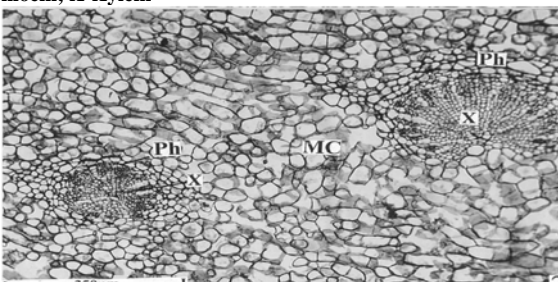


Fig. 6b: T.S of rind showing inner vascular bundle  
Magnified Ph- phloem; X-Xylem; MC- Mesocarp

Fig. 7: T.S of rind and septa (kabul variety)

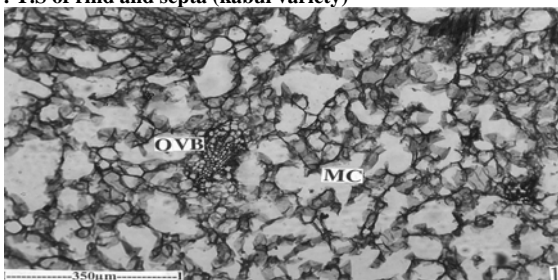


Fig. 7a: T.S of rind – outer smaller vascular bundle  
MC- Mesocarp; OVB - Outer vascular bundle

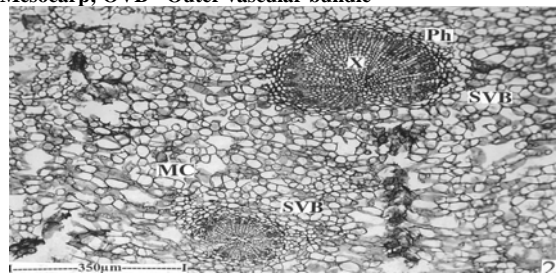


Fig. 7b: T.S of septa showing vascular bundle enlarged. SVB- Septa vascular bundle; Ph- phloem; X-Xylem; MC- Mesocarp;

Fig. 8: Anatomy of the septa (kabul variety)

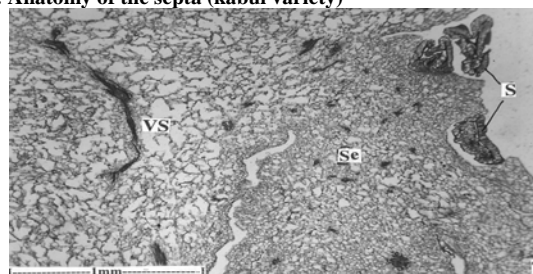


Fig. 8: T.S of Septa – a sector S- Seed; Se – Septa; VS – Vascular strand.

#### Microscopical examination of Ganesh variety rind

**Rind** of the fruit is 9 mm thick and it is yellowish red in color. It consists of cuticle, multiple epidermis and wide parenchymatous mesocarp.

**Cuticle** is thin and smooth. It covers the entire epidermal layer.

The **epidermis** consists of 2 – 4 layers small, squanish cells, which gradually transit in to a wide inner zone fairly wide, compact, four or five layers of parenchyma cells (Fig. 1-1a & 1b).The major inner portions of the mesocarp comprises of small, thin walled compact randomly arranged parenchyma cells. Scattered in the mesocarp are small nests of brachysclereids which are isodiametric sclerenchyma cells with very thick, lignified walls, wide lumen and canal like pits (Fig. 1-1a & 1b; Fig. 4b). The sclereids are 60-80µm long and 20-30 µm wide.

The **mesocarp** is well vascularised. There are many vascular strands of different shape and size scattered throughout the mesocarp (Fig 1b; 3a). The mesocarp extends towards the centre of the fruit in the form of fleshy irregular septa (Fig 2b). The septa consist of compact, thin walled parenchyma cells. No seeds were seen attached to the septa.

The **vascular strands** of the mesocarp are smaller in size in the peripheral zone and those in the inner zone are fairly large (Fig. 1b; 3a & 3b). The vascular strands are collateral, having xylem in inner zone and phloem situated along the outer zone. The xylem elements are wide, squanish Radially long; they are arranged in regular parallel longitudinal lines. The elements are 10 µm wide (Fig. 3a & 3b).

**Starch grains** aggregated in large masses in the inner part of the mesocarp, small circular and are and measured 5 µm in diameter.

#### Microscopical examination of Kabul variety rind

The **rind** is 1.2 mm thick and it is red in color.

The **epidermis** and the outer zone of the **mesocarp** are darkly stained due to heavy accumulation of tannins (Fig. 5a & 5b). Tanniniferous outer mesocarp cells were small, fairly thick walled and polygonal in shape. Tannin accumulation was not found in the inner mesocarp, cells were larger thin walled and loosely arranged having small intercellular spaces (Fig. 5b; 6b).

The **sclereids** and **vascular strands** were abundant in all regions of pericarp. Sclereids masses of varying sizes were scattered in the ground tissue and of brachysclereid type with circular to angular in outline; walls were thick and lignified. Some of them have wide lumen while others have narrow reduced lumen (Fig. 5b, 7) with a size of up to 50µm.

**Mesocarp** extends towards the centre form thick sheats with fringed margin. Shrunken and lobed seeds were seen attached at the inner part of the septa (Fig. 8). **Vascular strands** were scattered and well developed in mesocarp. In the peripheral

zone, vascular strands were fan shaped and expanded widely along the lateral part. Wide band of xylem consists of compact, narrow parallel elements which are angular, thick walled and lignified. Phloem occurs in small group along the narrow outer zone (Fig. 6a).

Towards the inner part of the mesocarp, vascular strands become nodular and have circle of xylem elements, ensheathed by a thin layer of phloem (Fig. 6b). Xylem cylinder consists of narrow, thick walled angular elements arranged in parallel lines and diverging towards the periphery. It seems that wide tangential band of vascular strands are broken into small units and were modified in to semi circles and circles due to pressure produced by the proliferating parenchymatous ground tissues and wide air-chambers.

**Starch grains** were not evident in the mesocarp tissue in addition to crystals.

## CONCLUSION

In conclusion, in both the transverse section of the rinds we observed the presence of epidermis, mesocarp, vascular strand, and cuticle. In the inner vascular bundle mesocarp were observed. Occurrence of phloem and xylem in the outer vascular bundle in both the rind varieties and starch grains of enormous amount were observed in septal region of Ganesh variety.

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