Research Article

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COMPARISON OF THE LEAF DRUG *RUTA GRAVEOLENS* AND ITS SUBSTITUTE *RUTA CHALEPENSIS*

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Received on: 25/08/18 Accepted on: 25/10/18

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DOI: 10.7897/2277-4343.096180

ABSTRACT

Ruta graveolens is known as common rue, belongs to family Rutaceae. Rue oil is obtained from steam distillation of fresh plant material and used as anthelminthic, antispasmodic, antiepileptic, rubefacient and emmenagogue. It's a potential drug used in all the traditional systems of medicine. *Aruvatha mattirai* and *Aruvathac curanam* are the Siddha formulations prepared from dried leaves of *R. graveolens*. The leaves of *R. chalepensis* commonly known as fringed rue, is reported to be used as a substitute owing to similar morphology in dried form. So this current study is to compare and contrast the authentic drug, *R. graveolens* from its substitute. Methods: Dried leaves of both species were collected from medicinal garden of Siddha Medical College, Arumbakkam. The macroscopic and microscopic details including powder were studied following the standard pharmacopoeial procedures. Results: Macroscopically leaves are different colored with dissimilar shape, in microscopic study *R. graveolens* showed toothed lamina with glandular trichomes while *R. chalepensis* showed lamina without trichomes. Quantitative microscopy of both species was carried out and the epidermal number, stomatal number, stomatal index, vein islet number, vein islet ratio, palisade ratio was recorded. Powder microscopy of *R. graveolens* leaves showed the presence of pitted and spiral vessels while *R. chalepensis* showed annular and spiral vessels. Conclusion: The study provides the characteristics features to distinguish *R. graveolens* from *R. chalapensis*.

Keywords: Adulteration, Powder microscopy, quantitative microscopy, Substitution

INTRODUCTION

Ruta graveolens L. is indigenous of Southeastern Europe but is widely naturalized in Southern Europe and cultivated worldwide. It is a perennial shrub with smooth erect stems; leaves are green colored compound leaves with lobed obovate leaflets, flowers are found in terminal umbels with four spoon-shaped yellow shining non- fringed petals; fruit is a capsule containing many seeds[1]. *R. graveolens* used in traditional medicines for the relief of pain, eye problems, rheumatism and dermatitis for a long time. Medicinal properties like anti-inflammatory, analgesic, antibacterial, antidiabetic[2] and antifertility[3] effects of *R. graveolens* extracts have been reported in various studies. The phytochemicals being discovered and isolated from the extracts are rutin, quercetin, rutacridone, gravacridondiol and the essential oil contains monoterpenes like limonene, α-pinene and 1,8-cineole[4]. In Siddha leaves of *Ruta graveolens* are used in the preparation of *Aruvatha mattirai* and *Aruvathac curanam*[5]. The plant is not mentioned in the classical Ayurveda books but described for cough, cold and dysmenorrheal in late nighantus of 20th century.

Ruta chalepensis is indigenous to Mediterranean region and Canary islands[6] is found growing on rocky places, dry banks and thickets, usually found on limestones[7] and is acclimatized world over. It is a perennial herb growing up to 80 centimeters tall. The leaves are compound; the inflorescence is a cluster of flowers, each with four or five bright yellow petals with rolled, fringed edges. The fruit is a textured capsule which is divided into pointed lobes. The plant is abortifacient, anthelmintic, emmenagogue and ophthalimic[7]. *R. chalepensis* is a rich source of important secondary metabolites such as furanocoumarins and alkaloids. Phytochemical screening of the aerial parts of the plant showed the presence of alkaloids, flavonoids, coumarins, tannins, volatile oil, sterols and triterpenes[8]. The pharmacological activities like antioxidant, anticancer and anti-inflammatory effects are reported from this species[9].

All *Ruta* species are associated with phyto photodermatitis[10] and plants should not be touched with bare hands, especially on sunny days. In 14 species of genus *Ruta*; *R. graveolens* and *R. chalepensis* are available in India and also cultivated in gardens. The plants are explored very less for its medicinal properties. Even though the taxonomical characters to recognize the Indian species are very apparent in case of flower morphology, references to it are mystified in the traditional literature[11]. So the anatomical study of the aerial parts of the plant can be a solution to mitigate this drawback for the proper identification and authentication of the plants since both are used in traditional systems of medicines, the adulteration or substitution of drugs can prevent to some extent.

MATERIALS AND METHODS

Botanically identified and authenticated leaves of *Ruta graveolens* (Voucher No: SCRIP010) and *Ruta chalepensis* (Voucher No: SCRIP011) were procured from the medicinal garden of Siddha Medical College, Arumbakkam. The macroscopy of the samples were documented by Nikon COOLPIX5400 digital camera. Sectioning of the fresh samples were done to reveal the anatomy of the parts and the rest were dried, powdered, passed through mesh no.60, and preserved in an air-tight covers for powder microscopy. Transverse sections of the leaves 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. Quantitative studies were carried out according to standard procedure. Magnifications were indicated by the scale-bars. A pinch of the powdered leaves were mounted in glycerine on a clean microscopic slide. Slides were observed under Nikon ECLIPSE E200 trinocular microscope and diagnostic characters were identified, photographed and documented[12].

RESULTS AND DISCUSSIONS

Macroscopy

The leaves of *R. graveolens* are bluish green colored when fresh and turn to yellowish brown when dried; leaves alternate, entire and fern like compound with deeply lobed obovate leaflets, gland dotted with dark spots; unipinnate to tripinnate; leaflets are linear to oval or oblong; 7.5 to 20 cm long and 2-3cm broad; leaves become fragile on drying and mostly found as powdery in the bulk sample. The odor is strongly aromatic and tastes slightly bitter.

In *R. chalepensis* the leaves are, grey- green colored, 10 to 12 cm long and 5 to 8 cm broad, compound and each divided into several segments which are subdivided into smaller leaflets; blong-elliptical to linear shaped leaflets with prominent dark spots and oil glands. The leaves have a strong and unpleasant smell tasting bitter (Fig. 1).

Microscopy

Petiole

TS of petiole of *R. graveolens* is circular in outline with outer epidermis followed by single layered hypodermis. The outer cortex consists of 3 to 4 layers of chlorenchyma cells with few volatile oil cells distributed randomly. The middle cortex is formed of 4 to 5 layers of collenchymas cells some of which contains prismatic crystals and rosette crystals. The inner cortex is made up of few parenchyma cells and a ring of 5 conjoint, collateral vascular bundles at the center. A discontinuous pericycle layer surrounds the vascular ring which is followed by a group of phloem cells, and xylem facing towards pith. The pith is formed of parenchyma cells (Fig. 2).

In *R. chalepensis*, the petiole appears circular in TS with single layered epidermis followed by a layer of hypodermis. The outer cortex is large and formed of 8 to 10 layers of chlorenchyma cells with randomly distributed volatile oil cells. The middle cortex is made up of few layers of collenchyma cells containing rosette crystals. A ring of 4 to 5 collateral vascular bundles are seen in the inner cortical region similar to that of *R. graveolens*. Parenchyma cells in the center forms the pith (Fig. 3).

Lamina

The TS of leaf of *R. graveolens* showed both upper and lower epidermis with cuticle followed by mesophyll cells. The epidermis bears glandular trichomes on both surfaces giving a toothed appearance to the epidermal layer. The mesophyll cells consist of two layers of palisade cells and loosely arranged spongy cells. Intervening the palisade layer some volatile oil cells is present. In between palisade and spongy layers a good number of rosette crystals of calcium oxalates are also found. The vasculature is formed of xylem and phloem elements (Fig. 4).

In *R. chalepensis*, the outer thick walled epidermis is single layered without having any trichomes. The mesophyll differentiated into upper double layered palisade and lower spongy parenchyma cells which are organized loosely. In the midrib region a layer of few cells of palisade also found on the lower epidermal region just below the vascular bundle. Volatile oil cells can be seen on the palisade layer. Abundance of rosette crystals of calcium oxalate is distributed in the lamina. The vascular bundle is formed of normal elements (Fig. 5).

Quantitative microscopy

The leaves of both species were subjected to quantitative microscopy. It revealed the presence of peltate glandular trichomes on both the surfaces of *R. graveolens* leaf and abundance of rosette crystals on *R. chalepensis* leaf (Fig. 6 & 7).

Table 1: Quantitative microscopy of *Ruta graveolense* and *R. chalepensis*

	R. graveolens		***R. chalepensis***	
Parameters	**Upper (/mm^2)**	**Lower (/mm^2)**	**Upper (/mm^2)**	**Lower (/mm^2)**
Epidermal number	88 – 128	80 - 176	56 - 164	80 – 100
Trichome number	8 – 10	10 - 12	-	-
Stomatal number	40 – 54	48 - 60	40 - 52	52 – 76
Stomatal index	29 – 31	25 - 38	24 - 41	40 – 43
Palisade ratio	3.25 - 5		6 - 6.75	
Vein islets number	12 - 16		5 – 6	
Vein termination number	29 - 39		15 – 20	

Table 2: Comparison between *R. graveolens* and *R. chalepensis*

Characteristic features		***Ruta graveolens* L.**	***Ruta chalepensis* L.**
Macroscopy			
Leaves			
1.	Colour	Blue green	Grey-green
2.	Size	7.5 to 20 cm long, 2-3cm broad	10 to 12 cm long, 5 to 8 cm broad
3.	Shape	Obovate- linear to oval	Oblong- elliptical to linear leaflets
Flowers		Non-fringed petals	Fringed petals
Fruits		Capsule with blunted tips	Capsule with pointed tips
Microscopy			
Leaves			
1.	Lamina	Toothed surface	Smooth surface
2.	Trichomes	Glandular	Absent
3.	Rosette crystals	Scares	Abundant
Petiole			
1.	Cortex	Narrow cortex with 2 to 3 layers of chlorenchyma	Broad cortex with many layers of chlorenchyma
2.	Prismatic crystals	Present	Absent

Powder Microscopy			
1.	Colour	Brownish yellow	Yellowish brown
2.	Odour	Strongly aromatic	Unpleasant
3.	Taste	Slightly bitter	Bitter

Powder Microscopy

The leaf powder of *R. graveolens* was brownish yellow with characteristic aroma and tastes slightly bitter and showed characters like epidermis of petiole in surface view, leaf epidermis in surface view, cortical parenchyma of petiole, spongy parenchyma, pith parenchyma with vasculature, vessels with annular and pitted thickening, fibers with wide lumen and xylem parenchyma cells, vessel fragments and oil cavity cells (Fig. 8).

The leaf powder of *R. chalepensis* was yellow brown with unpleasant smell and bitter taste. It showed the characters like leaf epidermal cells in surface view, fragment of mesophyll cells, spongy parenchyma, pith parenchyma with vasculature, vessels with annular and spiral thickenings, thick walled fibers with wide lumen, rosette crystals, starch grains and oil globules (Fig. 9).

The two species of *Ruta* growing in India are very difficult to identify and differentiate since both are morphologically very similar and having almost same anatomical features with negligible differences. These were used interchangeably in ancient times[13] and now a day's *R. chalepensis* is become an adulterant for *R. graveolens*. The studies available in the literature is also confusing and contradicting each other and need a very clear description and characterization of the two for the authentication of plant species. The comparison of macro and microscopic characters are being supportive for the better discrimination between the two plants. The blue-green appearance of leaves in *R. graveolens* distinctly differentiates *R. chalepensis* with grey green leaves and fringed petals of *R. chalepensis* from non-fringed flowers of *R. graveolens.*

Presence of essential-oil cavities mentioned as the characteristic feature of Rutaceae[14] was present in random distribution in both. Even though these plants are gland dotted and scented, they show a distinct difference in the odor and taste. This study gives the key distinguishing character of presence of glandular trichomes in *R. graveolens* leaf which supports the works of Jayaweera[15] while R. Kannan et al., (2012)[16] strongly disagrees the findings that they couldn't observed the trichomes even in both species. But the photographs illustrated in the Fig 4.4 and 4.5 gives the greater evidence of occurrence of trichomes on both the leaf surfaces of *R. graveolens* giving it a toothed appearance. The stomatal diversity characteristics (type, shape, size and orientation) in the foliar epidermis have a great value in plants systematic and taxonomic studies[17]. The presence of anomocytic stomata in *R. graveolens* also reported in earlier records[18,19] but they reported the occurrence only on the lower surface. In this study we could observe obviously the amphistomatic distribution of stomata in both species which similar to the findings of Dhale et al., 2010[19].According to Metcafe and Chalk[20] palisade ratio is a reliable taxonomic character, which is constant for a taxon and it will not vary with environment. The palisade ratio we recorded is nearly same as findings of Dhale et al., 2010[19].The presence of broad outer cortex with chlorenchyma layers in the petiole can distinguish *R.chalepensis*from *R. graveolenswhere* only 2 to 3 layers are observed. The abundant of rosette crystals of calcium oxalates in leaves is also a significant character in *R. chalepensis* while comparing with that of scares amount in *R. graveolens*. But *R. graveolens*possess prismatic crystals in petiole whereas which is absent in *R. chalepensis.*

CONCLUSION

The pharmacognostic characters described to distinguish the *Ruta* species will be a supportive tool to compare the plants. Since most of the pharmacognostic publications pertaining to these plants are contradicting each other further DNA finger printing and molecular studies are necessary to accomplish the identity and authenticity of the species.

ACKNOWLEDGEMENT

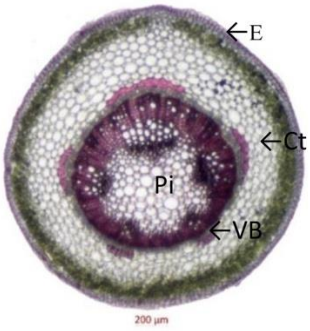
The authors extend their heartfelt thanks to Director General, Central Council for Research in Siddha, and Assistant Director, Incharge, Siddha Central Research Institute, Chennai for the support.

Figure 1: Macroscopy of *Ruta graveolens* and *Ruta chalepensis*

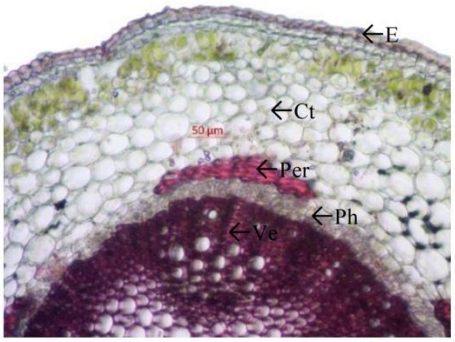
1.1 *R. graveolens*

1.2 *R. chalepensis*

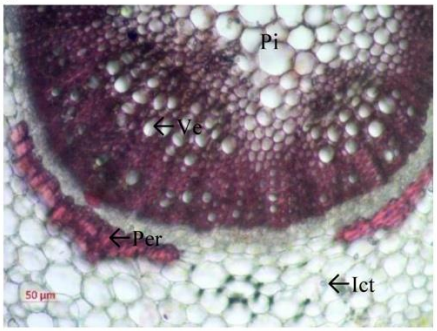
Figure 2: Microscopy of *Ruta graveolens* petiole



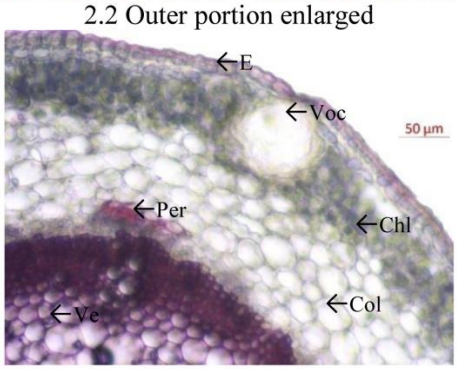
2.1 TS of petiole



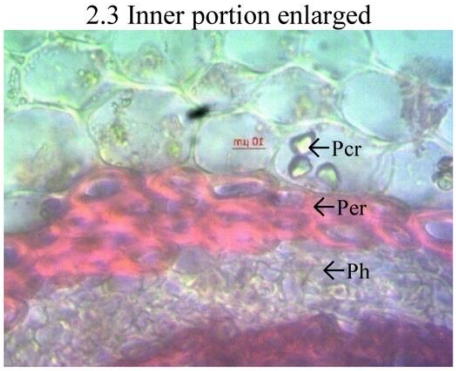
2.2 Outer portion enlarged



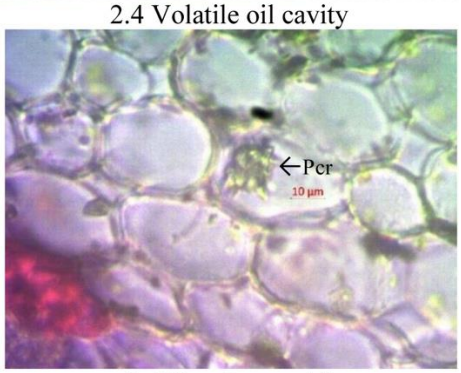
2.3 Inner portion enlarged



2.4 Volatile oil cavity



2.5 Pericycle and prismatic crystals

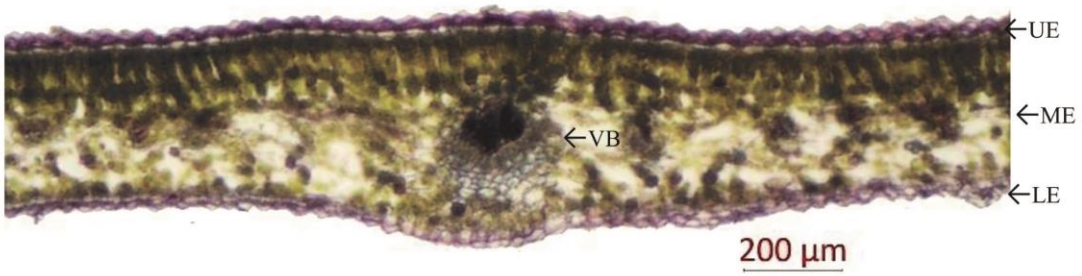


2.6 Rosette crystal

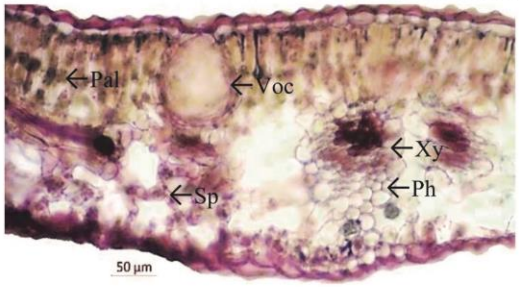
2.7 Xylem

E – Epidermis; Chl - Chlorenchyma; Col – Collenchyma; Ct – Cortex; E – Epidermis; Ict – Inner cortex; Pcr – Prismatic crystals; Per – Pericycle ; Ph – Phloem; Pi – Pith; VB – Vascular Bundle; Ve – Vessel; Voc – Volatile oil cell; Xy – Xylem.

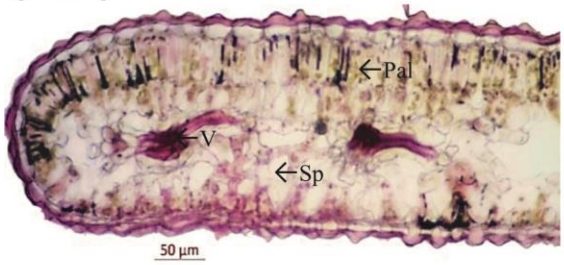
Figure 3: Microscopy of *Ruta graveolens* leaf



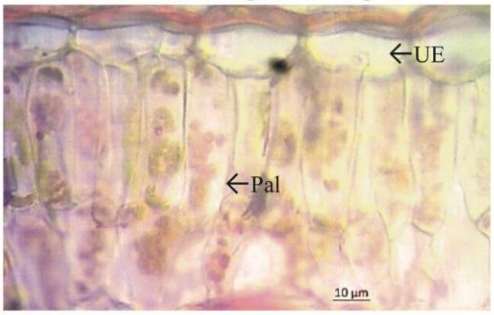
3.1 TS of lamina passing through midrib



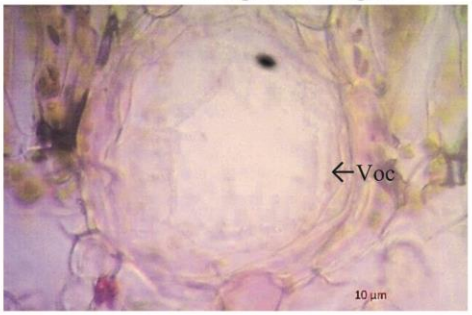
3.2 Midrib region enlarged



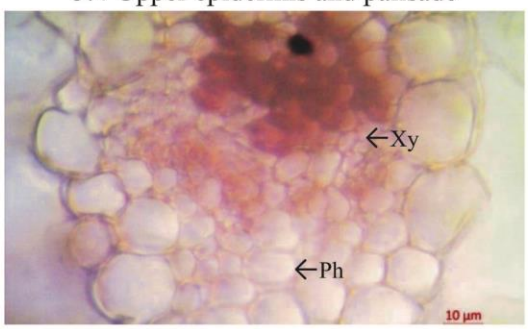
3.3 Lamina region enlarged



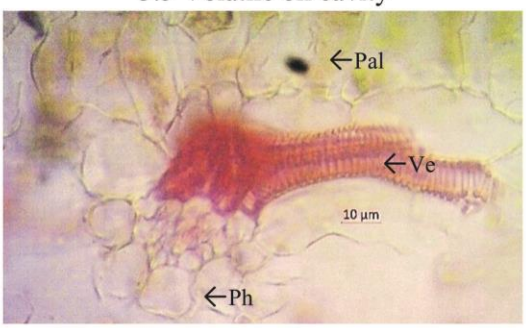
3.4 Upper epidermis and palisade



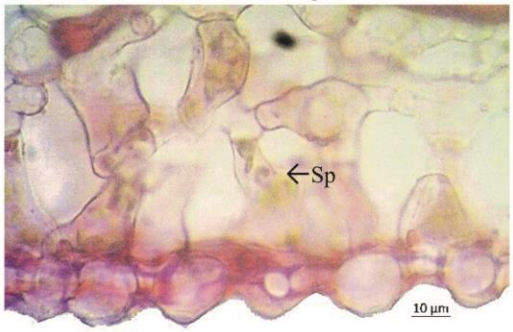
3.5 Volatile oil cavity



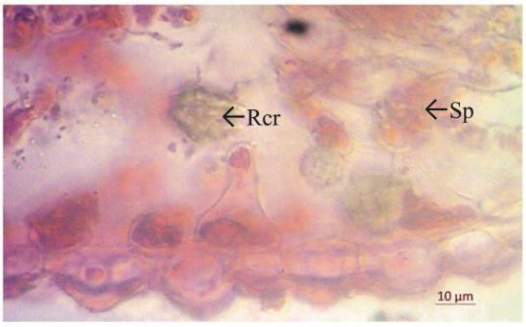
3.6 Midrib region



3.7 Vascular bundle



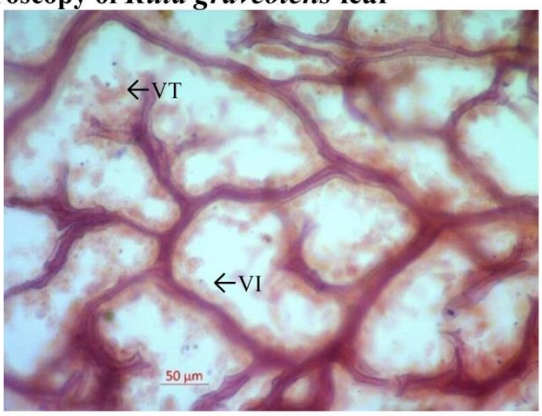
3.8 Lower epidermis and spongy parenchyma



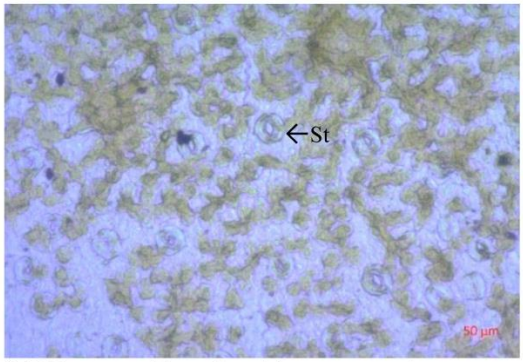
3.9 Rosette crystals

LE – Lower Epidermis; Me – Mesophyll; Pal – Palisade cells; Ph – Phloem; Rcr – Rosette crystals; Sp – Spongy parenchyma; UE – Upper Epidermis; V – Vein; VB – Vascular Bundle; Ve – Vessel; Voc – Volatile oil cell; Xy – Xylem.

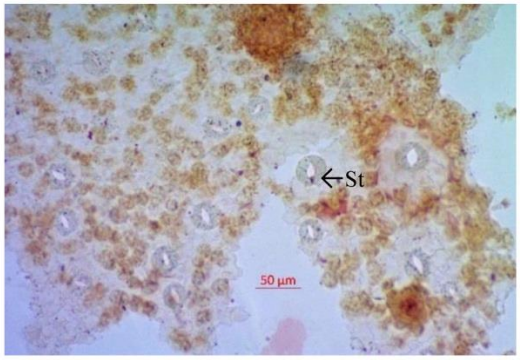
Figure 4: Quatnitative Microscopy of *Ruta graveolens* leaf



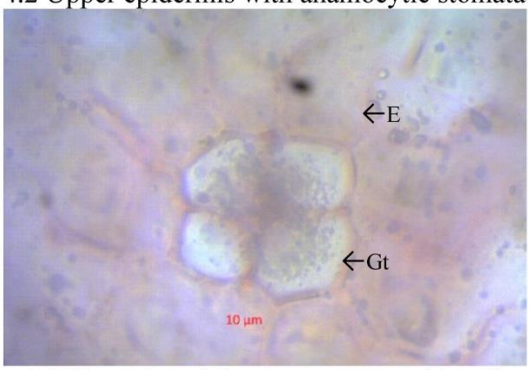
4.1 Vein Islets and vein terminations



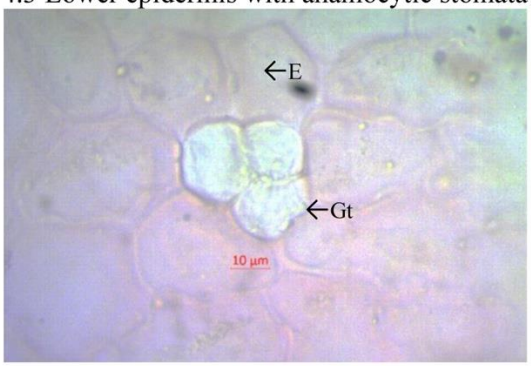
4.2 Upper epidermis with anamocytic stomata



4.3 Lower epidermis with anamocytic stomata

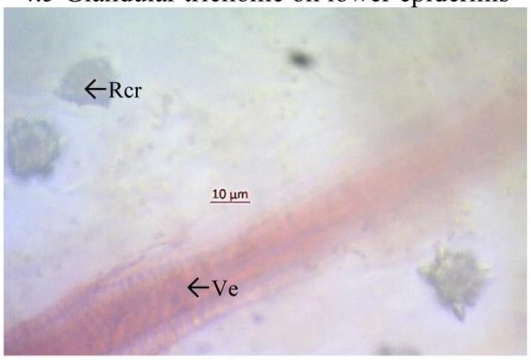


4.4 Glandular trichome on upper epidermis



4.5 Glandular trichome on lower epidermis

4.6 Stomata enlarged view

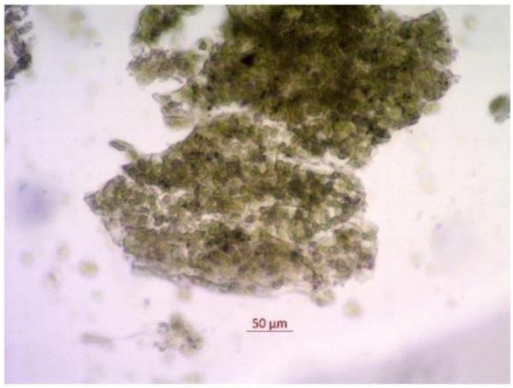


4.7 Rosette crystals

E – Epidermis; GT – Glandular Trichome; St – Stomata; VI – Vein islet; VT – Vein termination

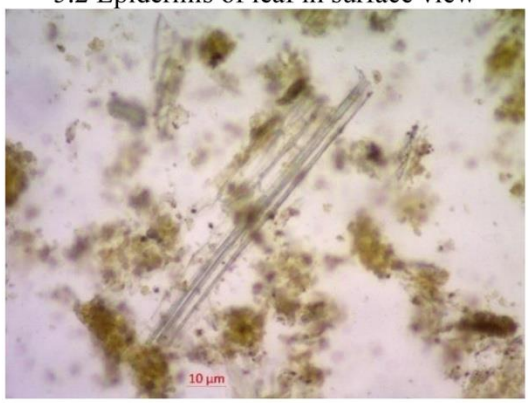
Figure 5: Powder Microscopy of *Ruta graveolens* leaf

5.1 Epidermis of petiole in surface view

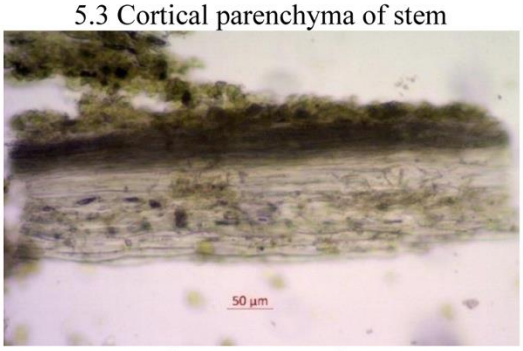


5.2 Epidermis of leaf in surface view

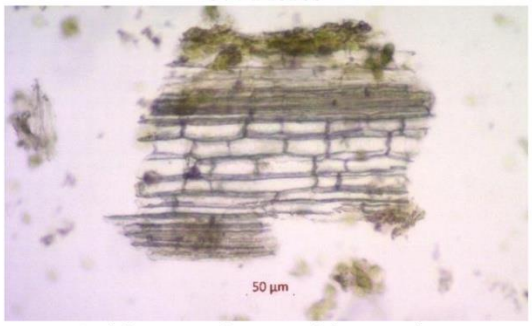
5.3 Cortical parenchyma of stem



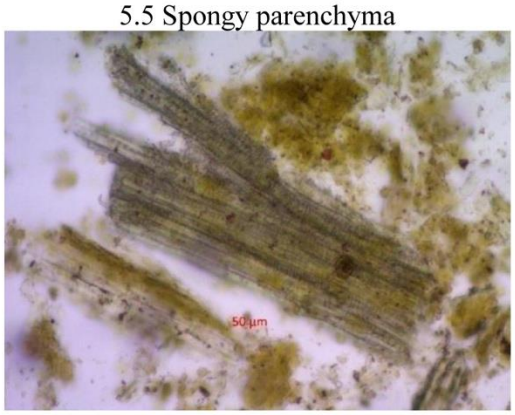
5.4 Fibres



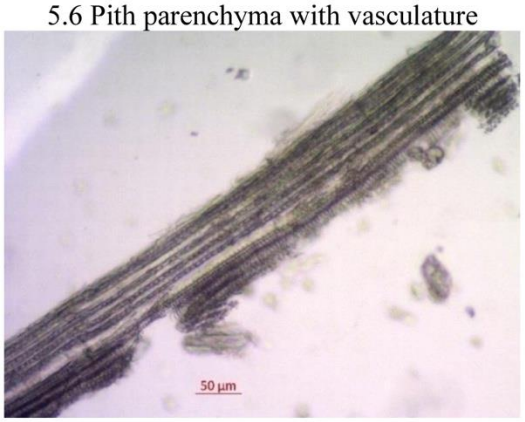
5.5 Spongy parenchyma



5.6 Pith parenchyma with vasculature

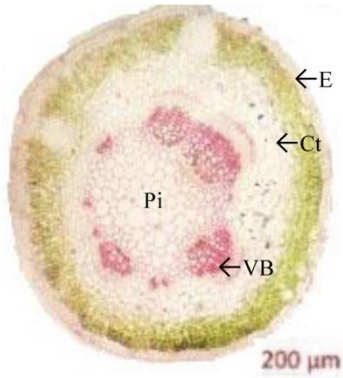


5.7 Vessel fragments and oil cavity

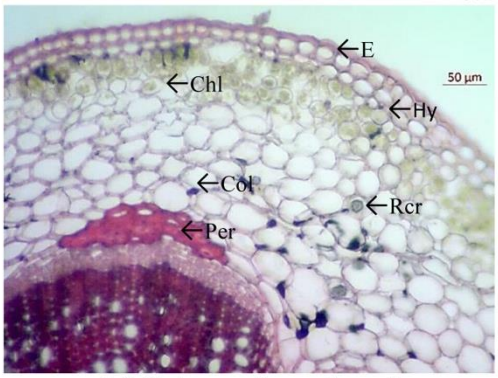


5.8 Xylem with pitted and spiral vessels

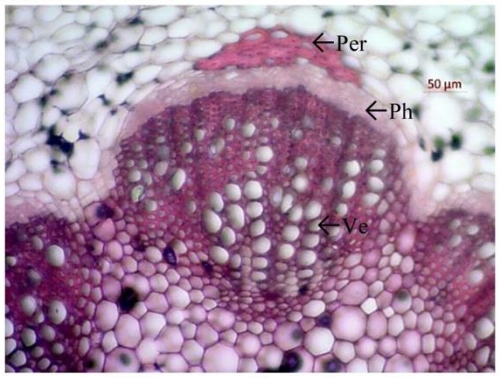
Figure 6: Microscopy of *R. chalepensis* petiole



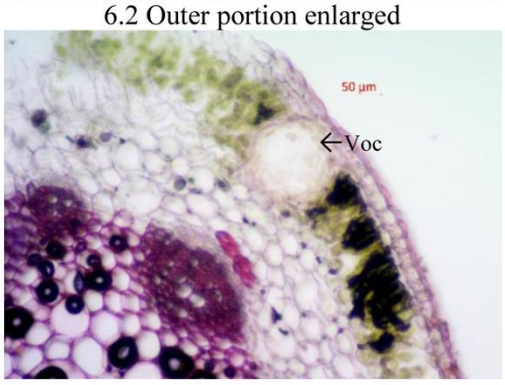
6.1 TS of petiole



6.2 Outer portion enlarged

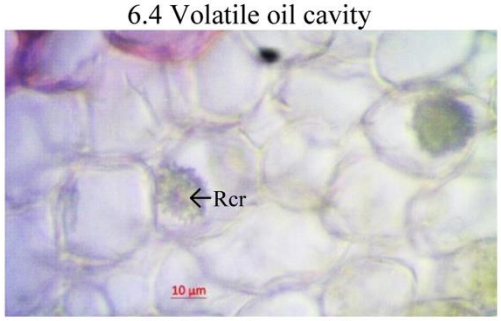


6.3 Inner portion enlarged

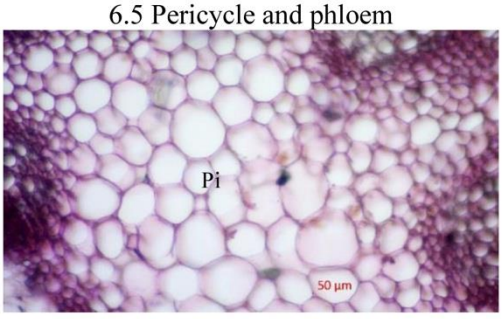


6.4 Volatile oil cavity

6.5 Pericycle and phloem



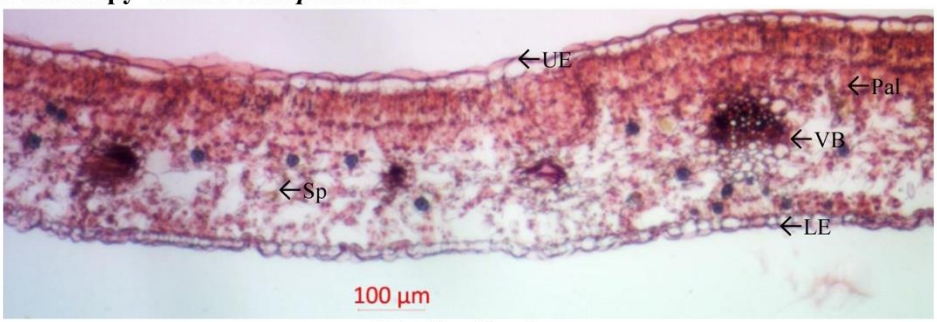
6.6 Rosette crystals



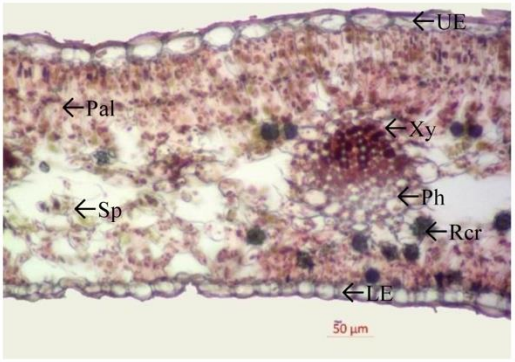
6.7 Pith region

E – Epidermis; Chl - Chlorenchyma; Col – Collenchyma; Ct – Cortex; E – Epidermis; Hy – Hypodermis; Pcr – Prismatic crystals; Per – Pericycle; Ph – Phloem; Pi – Pith; VB – Vascular Bundle; Ve – Vessel; Voc – Volatile oil cell; Xy – Xylem.

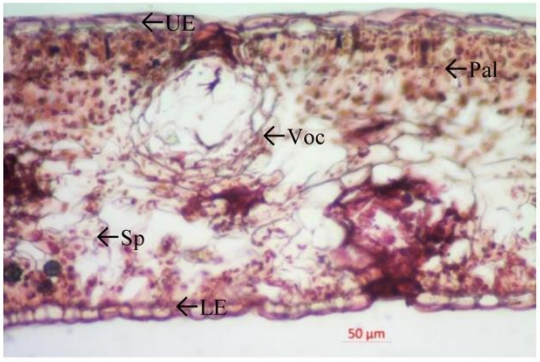
Figure 7: Microscopy of *Ruta chalepensis* leaf



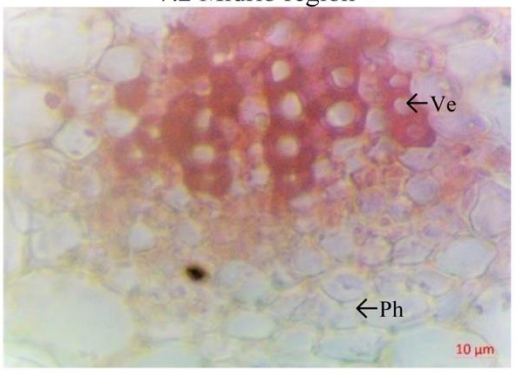
7.1 TS of lamina



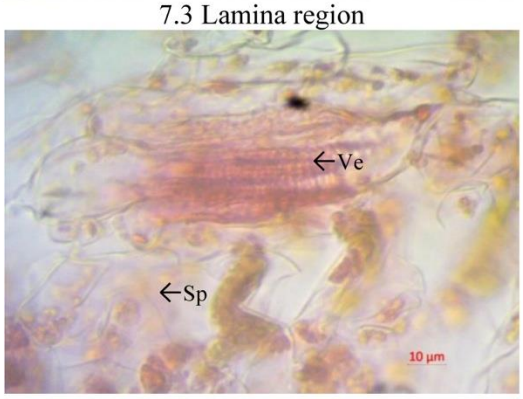
7.2 Midrib region



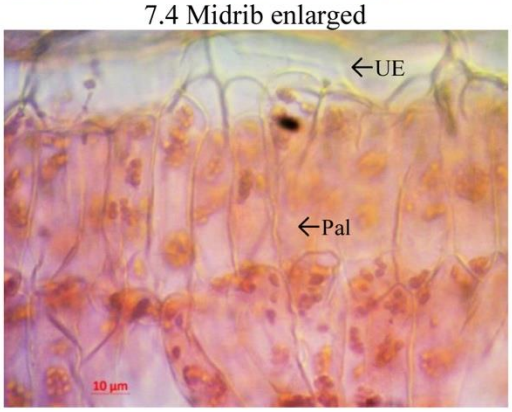
7.3 Lamina region



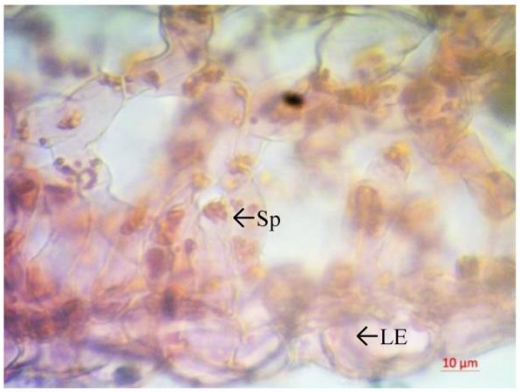
7.4 Midrib enlarged



7.5 Vascular bundle



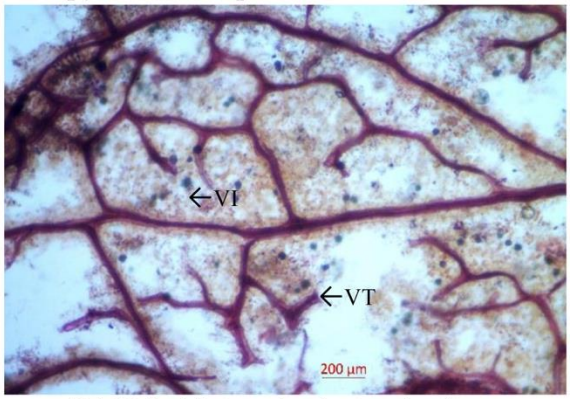
7.6 Upper epidermis and palisade



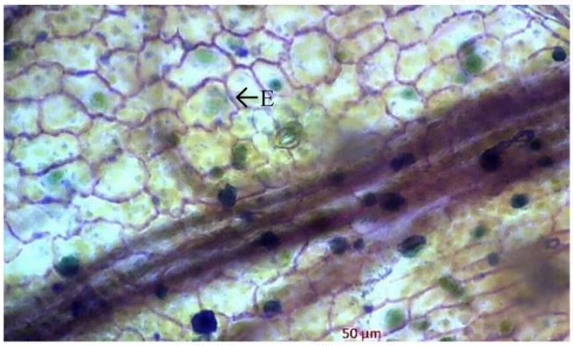
7.8 Lower epidermis and spongy parenchyma

LE – Lower Epidermis; Me – Mesophyll; Pal – Palisade cells; Ph – Phloem; Rcr – Rosette crystals; Sp – Spongy parenchyma; UE – Upper Epidermis; V – Vein; VB – Vascular Bundle; Ve – Vessel; Voc – Volatile oil cell; Xy – Xylem.

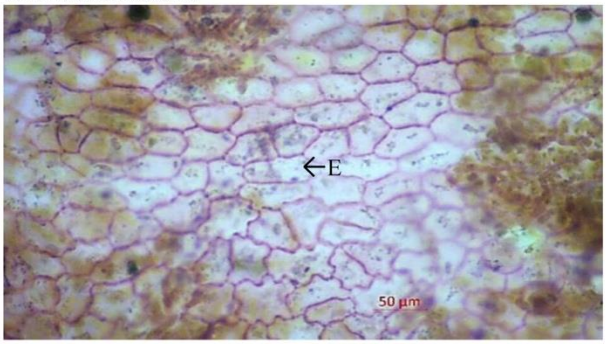
Figure 8: Quatnitative Microscopy of *R. Chalepensis* leaf



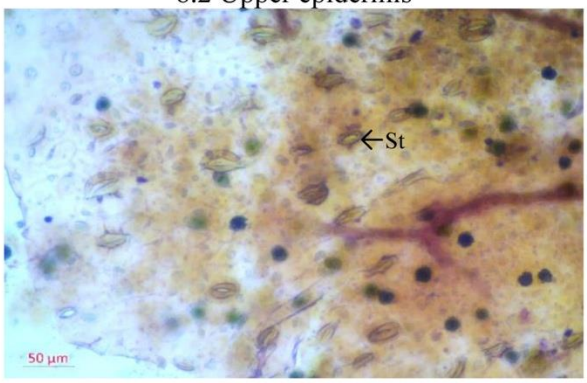
8.1 Vein islets and vein terminations



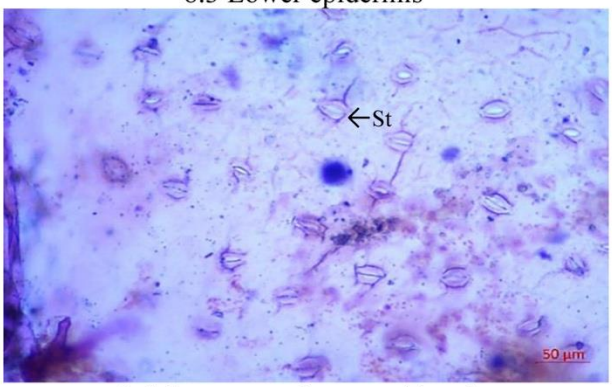
8.2 Upper epidermis



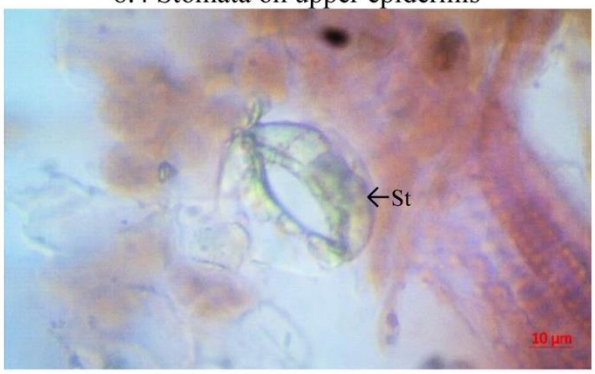
8.3 Lower epidermis



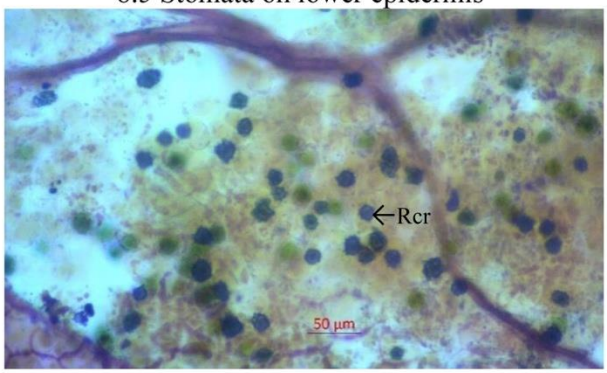
8.4 Stomata on upper epidermis



8.5 Stomata on lower epidermis



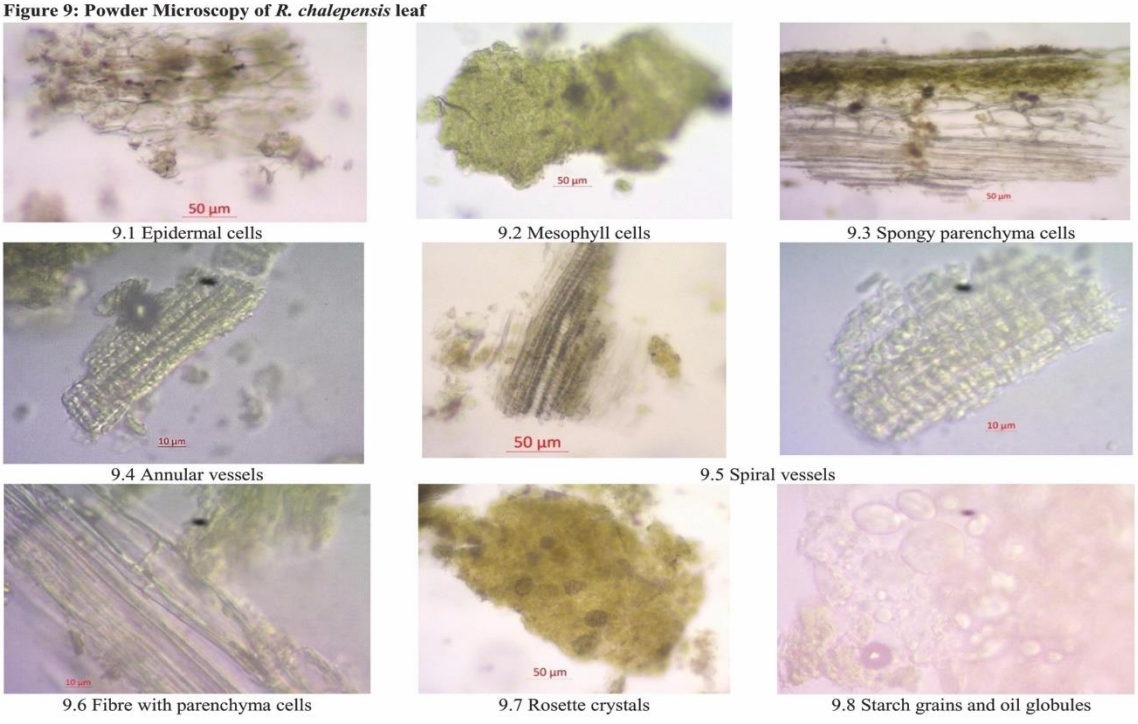
8.6 Stomata enlarged view



8.7 Rosette crystals

E – Epidermis; St – Stomata; VI – Vein islet; VT – Vein termination

Figure 9: Powder Microscopy of *R. chalepensis* leaf



9.1 Epidermal cells | 9.2 Mesophyll cells | 9.3 Spongy parenchyma cells

9.4 Annular vessels | 9.5 Spiral vessels

9.6 Fibre with parenchyma cells | 9.7 Rosette crystals | 9.8 Starch grains and oil globules

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Cite this article as:

Rubeena Mattummal *et al*. Comparison of the leaf drug *Ruta graveolens* and its substitute *Ruta chalepensis*. Int. J. Res. Ayurveda Pharm. 2018;9(6):100-110 http://dx.doi.org/10.7897/2277-4343.096180

Source of support: Nil, Conflict of interest: None Declared