ORIGINAL ARTICLE

Evaluation of pharmacognostical changes in the mature and tender leaves of *Panchapallava* (group of five leaves), described in Ayurveda

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Abstract

Background: Ayurveda describes certain drugs under the heading *Mishrakavarga* (group of drugs) for therapeutic applications. *Panchapallava* (group of five leaves), one of these groups, is the combination of leaves of *Amra* (*Mangifera indica* L.), *Jambu* (*Syzygium cumini* (L.) Skeels), *Kapittha* (*Feronia limonia* (L.) Swingle), *Bijapura* (*Citrus medica* L.), and *Bilva* (*Aegle marmelos* (L.) Correa), is used in the management of *Atisara* (diarrhea), *Grahani* (sprue syndrome) and for the processing of many drugs. The technical term pallava indicates tender leaves but routine in pharma practice; mature leaves are used due to unavailability of tender leaves across the year. **Aim and Objectives:** The present study has been conducted to evaluate the similarities and difference if any, in cellular content and other pharmacognostical characters (morphological along with microscopical) of the tender and matured leaves of *Panchapallava* (group of five leaves). **Materials and Methods:** Freehand transverse sections of the matured and tender leaves through midrib were taken and examined under a microscope to locate various constituents' *viz*. oil and tannin content along with the micrometric evaluation of secretory cavity and resin canals following standard procedures. **Result and Conclusion:** The tender and matured leaves showed some common characters such as secretory cavity, tannin content, crystals, and oil globules. The size of the secretory cavity was found more in mature leaves than the tender leaves, whereas the oil globules, all over the cells, were found more in tender leaves than the mature leaves.

Key word: *Amra*, *Jambu*, *Kapittha*, *Bijapura*, *Bilva*, Leaf, Pharmacognosy, *Gandhakarma*

INTRODUCTION

Ayurveda describes many group of drugs under the heading *Mishrakavarga* based upon either certain pharmacognostical or pharmacological properties such as *Triphala* (group of three fruits), *Trikatu* (group of three pungent herbs), and *Panchatikta* (group of five bitters herbs) for therapeutic applications. *Panchapallava* (group five young leaves), one of these *Mishrakavarga* groups, is the combination of leaves of *Amra* (*Mangifera indica* L.; Anacardiaceae), *Jambu* (*Syzygium cumini* (L.) Skeels; Myrtaceae), *Kapittha* (*Feronia limonia* (L.) Swingle; Rutaceae), *Bijapura* (*Citrus medica* L.; Rutaceae), and *Bilva* (*Aegle marmelos* (L.) Correa; Rutaceae).[1] *Panchapallava* has been indicated in the management of *Atisara* (diarrhea), *Grahani* (irritable bowel syndrome), and *Mukhapaka* (stomatitis). *Panchapallava* is also mentioned to use in *Sugandhakarma* (aromatic purpose) and for the purpose of *shodhanakarma* (purificatory procedures) of *Vacha* (*Acorus calamus* L.), *Mustha* (*Cyperus rotundus* L.), etc.[2-4] However, the rationality behind selection only a few drug into certain groups have not been elaborated in any of the classical texts. There are many hypothesis developed by different authors of Dravyaguna text of present era.[5,6]

In taxonomy, these five plants belong to three families, i.e., Rutaceae, Anacardiaceae, and Myrtaceae. There are

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many similar characteristic features which help to bring the plant of these 3 families in a category to make a group. The members of the family Anacardiaceae is mostly, tree or shrub. The outstanding anatomical feature is the occurrence of resin canals in the region of phloem. The presence of pericyclic fibers surrounding the vascular bundle, solitary and cluster crystal and hypoderm, etc. are the reported identifying characters of the family Anacardiaceae.[7]

The member of the family Rutaceae is mostly tree or shrub. Trichomes are frequent in many members of this family. The ground tissue of both leaf and axis is nearly always characterized by the presence of secretory cavities. One or more layers of hypoderm, sometimes locally distributed occur in many species; signify the identifying characters of the family Rutaceae.[8]

The member of the family Myrtaceae is mostly tree or shrub. One of the important diagnostic characters of this family is the presence of secretory cavities, which are lined with epithelium when young and almost invariably secrete oily substances. Hypoderm below upper epidermis and presence of solitary and cluster crystals are also important characters of the plants, belongs to this family.[9]

The term *Panchapallava* furnished by two words, i.e., *Pancha* (five) and *Pallava* (tender leaf). On this account, it can be inferred that the tender leaves of these five plants should be used in the name of *Panchapallava*. In pharma practice, both mature and tender leaves are used due to the lack of the availability of tender leaves through the entire year. The Recent review reveals that there is no published literature to confirm whether to take mature or tender leaves with proper scientific background. The previous study highlighted on the fact that growth stages and seasons show effect on ecophysiological parameters of the plant. It was found that the shoot of *Brahmi* (*Bacopa monnieri* (L.) Penn.) have the highest saponin content compared with lower parts and roots.[10,11] The previous study also revealed that leaves of *Haritaki* (*Terminalia chebula* Retz.) before flowering-fruiting are having a more concentration of tannin than after flowering-fruiting leaves.[12]

Hence, the present study has been conducted to evaluate the similarity and differences of cellular and phytoconstituents along with other pharmacognostical characters of both tender and mature leaves of these five plants.

MATERIAL AND METHODS

Materials

Selection and collection of leaves

Tender and matured leaves of all the five plants, i.e., *Amra* (*M. indica* Linn.), *Jambu* (*S. cuminii* (Linn.) Skeels), *Kapittha* (*F. limonia* (Linn.) Swingle), *Bijapura* (*C. medica* Linn), and *Bilva* (*A. marmelose* (L.) Correa ex Roxb.) were collected by the first author (scholar) from the natural habitat of Jamnagar in the month of July 2014.

Chemicals

Sudan III and FeCl3 were obtained from Sigma-Aldrich. Mumbai, India and other reagents or chemicals used were of standard grade or analytical grade.

Methods

Morphological study

Leaves characters such as shape, size, margin, apex, attachment were scientifically studied as per taxonomy and verified with existing floras for authentication.[13,14]

Microscopical study

Freehand transverse sections of five leaves through midrib were taken. Sections were observed in distilled water under microscope. Microphotographs were taken using Carl zeiss trinocular microscope (Axiovision Rel 4.8.2) attached with camera.[15]

Localization of different constituents

Sections were stained with Sudan III and ferric chloride solution and again examined to locate oil content and tannin content in the respective samples. The samples were observed under compound microscope (QUASMA, India) and photographs were taken using Kodak easy share C140, 8.2 megapixels 3x optical/5x digital zoom HD camera.[15,16]

Micrometric evaluation

Measurements of resin canals (*Amra*) and secretory cavities (*Jambu, Kapittha, Bijapura and Bilwa*) were taken into consideration with the help Carl Zeiss Trinocular microscope attached with a camera with preloaded micrometric analysis software.[16]

Surface Study

Both upper and lower surfaces of leaves were peeled out, washed with chloral hydrate, observed under microscope with distilled water for stomatal structure, distribution along with vein islet number per sq. mm of leaf.[17,18] Measurement of the length, breath of stomata were also taken into consideration for micrometric evaluation with the help of compound microscope(QUASMA, India) and photographs were taken by using Kodak easy share C140, 8.2 megapixels ×3 optical/×5 digital zoom HD camera.[19]

Powder Microscopy

Cut pieces of leaf were dried under shade, powdered with the help of mechanical grinder and sieved through mesh

no. 60. Leaf powder was studied under microscope with distil water.[19]

RESULTS

Morphology

Amra (M. indica Linn.*)*

Tree, Leaves variable in size and shape, petiolate, petiole 2-3 cm, convex beneath, grooved or flat above, tender leaf reddish in color and about 11 cm × 2.5 cm whereas mature leaf 22 cm × 4.4 cm, leaf base pulvinus, usually narrowly elliptic to lanceolate, margin entire, acute to acuminate apex [Figure 1a and b].

*Jambu (S. cuminii (*Linn.*)* Skeels*)*

Large tree leaves glabrous, simple, petiolate, oblong to elliptic, mature leaf about 14 cm × 5.1 cm and tender leaf 8 cm × 2 cm, tender leaf reddish in color, acute to acuminate apex, shining or glabrous, secondary nerves meeting near the margin known as intramarginal nerve [Figure 2a and b].

*Kapittha (F. limonia (*Linn.*)* Swingle*)*

Tree with axillary spines leaves 5-7 foliate (compound), petiole narrowly winged, mature leaf about 6.9 cm × 4.4 cm and tender leaf 2.1 cm × 2.7 cm in dia. leaflet glabrous, subsessile, obovate, entire with obtuse apex, terminal one larger, mature leaflet about 3.4 cm × 2 cm, and tender leaflet 1.4 cm × 0.8 cm [Figure 3a and b].

Bijapura (C. medica Linn.*)*

Shrub with spines, petiole 0.5-1 cm, slightly winged, leaflet ovate-elliptic, tender leaflet about 5.2 cm × 1.7 cm and mature leaflet 11.7 cm × 5.7 cm, margin crenate to serrulate, glabrous, apex obtuse [Figure 4a and b].

Bilva (A. marmelose (L.) Correa ex Roxb.*)*

Tree with axillary spines, leaf 3 foliate (compound), petiolate 1.5-2 cm, mature leaf about 6.7 cm × 7 cm and tender 4 cm × 3.5 cm, leaflets ovate to lanceolate, terminal one larger, entire with acute to acuminate apex, petiolule 1-2 mm and about 1 cm of the terminal one, mature leaflet 4.6 cm × 2.2 cm and tender leaflet 2.5 cm × 0.8 cm [Figure 5a and b].

Microscopic Evaluation (Transverse Section)

M. indica Linn.

Tender

The diagrammatic sketch showed that leaf, dorsiventral with upper palisade and lower spongy parenchyma cells. Midrib showed centrally located vascular bundle. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermial cells with cuticle. Some

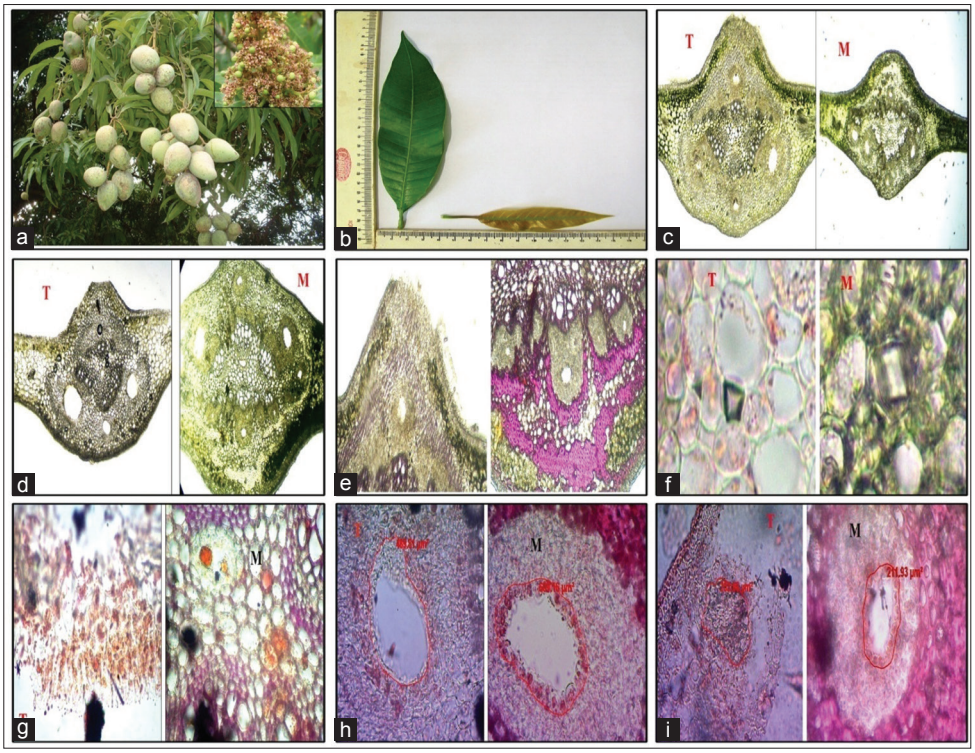

Figure 1: Morphology and microscopy of *Mangifera indica* Linn (*Amra*). (a) Whole plant photo, (b) Measurement of tender and mature leaf, (c) T.S. of midrib through lamina (×10), (d) T.S. after staining with $FeCl_3$ (×10) (e) Pericyclic fiber (×40), (f) Prismatic crystals of calcium oxalate (×40), (g) Oil globules (×40), (h) Resin canal in midrib (×40), (i) Resin canal in lamina (×40) (T for Tender leaf; M for Mature leaf)

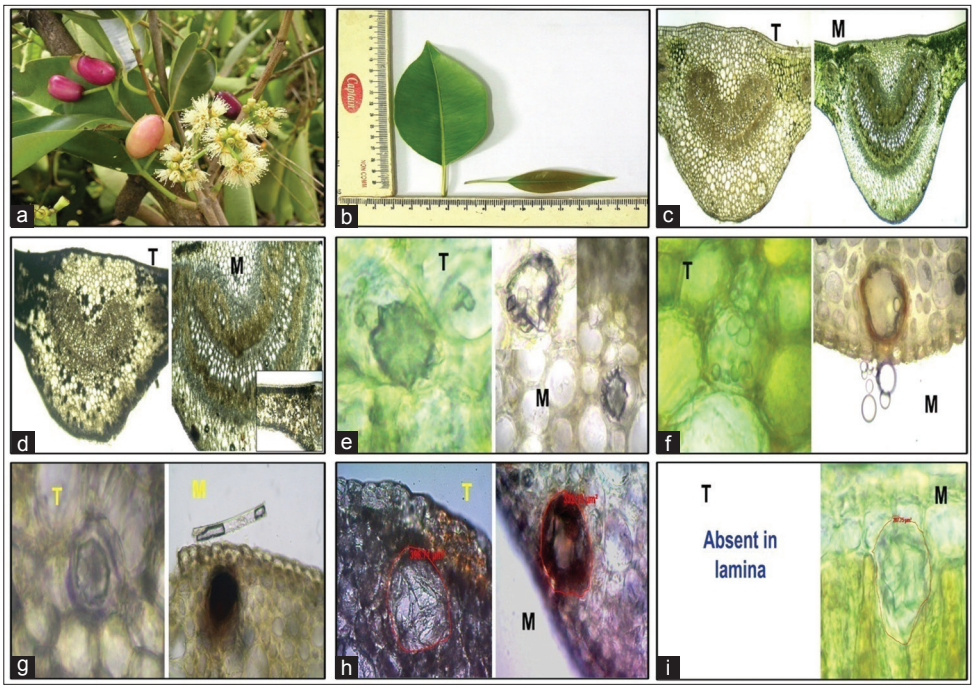

Figure 2: Morphology and microscopy of *Syzygium cuminii* (Linn.) Skeels (*Jambu*). (a) Whole plant photo, (b) Measurement of tender and mature leaf, (c) T.S. of midrib through lamina (×10), (d) T.S. after staining with $FeCl_3$ (×10), (e) Crystals of calcium oxalate (×40), (f) Oil globules (×40), (g) Cavity containing crystal and brown content (×40), (h) Secretory cavity in midrib (×40), (i) Secretory cavity in lamina (×40) (T for Tender leaf; M for Mature leaf)

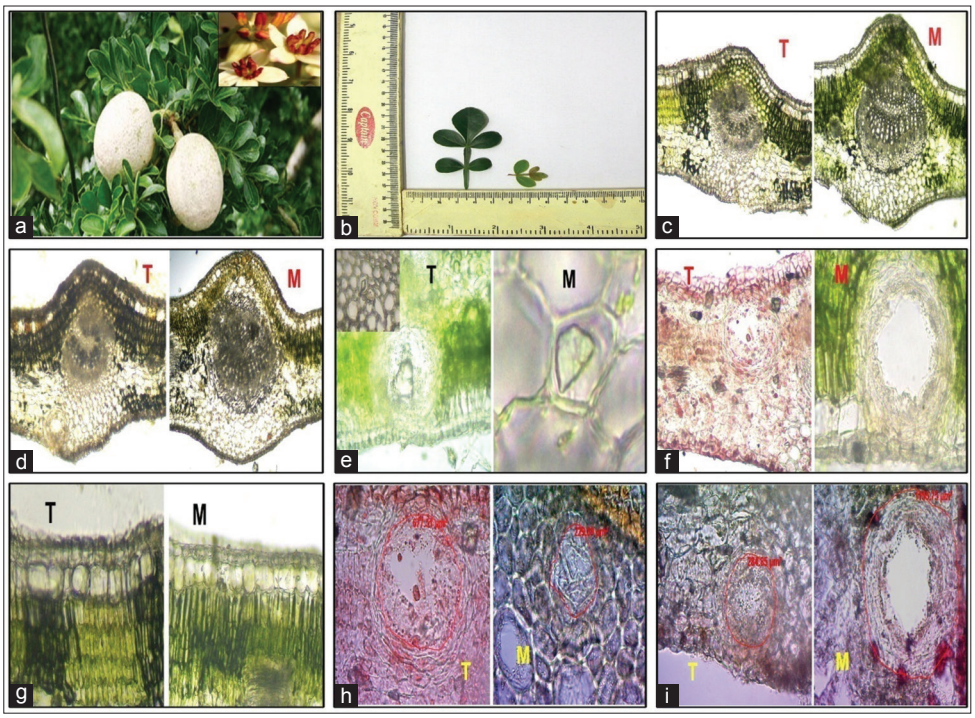

Figure 3: Morphology and microscopy of *Feronia limonia* (Linn.) Swingle (*Kapittha*). (a) Whole plant photo, (b) Measurement of tender and mature leaf, (c) T.S. of midrib through lamina (×10), (d) T.S. after staining with $FeCl_3$ (×10), (e) Crystals of calcium oxalate (×40), (f) Oil globules(×40), (g) Hypoderm layer (×40), (h) Secretory cavity in midrib (×40), (i) Secretory cavity in lamina(×40) (T for Tender leaf; M for Mature leaf)

of the epidermal cells interrupted by stomatal openings. Mesophyll consists of upper compactly arranged without any air spacing 1-2 layers of the elongated palisade and lower 5-6 layers of oval to rounded shaped with intercellular spaces, rich in chlorophyll pigment. Between the palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by large resinous canal consists oil and resin.

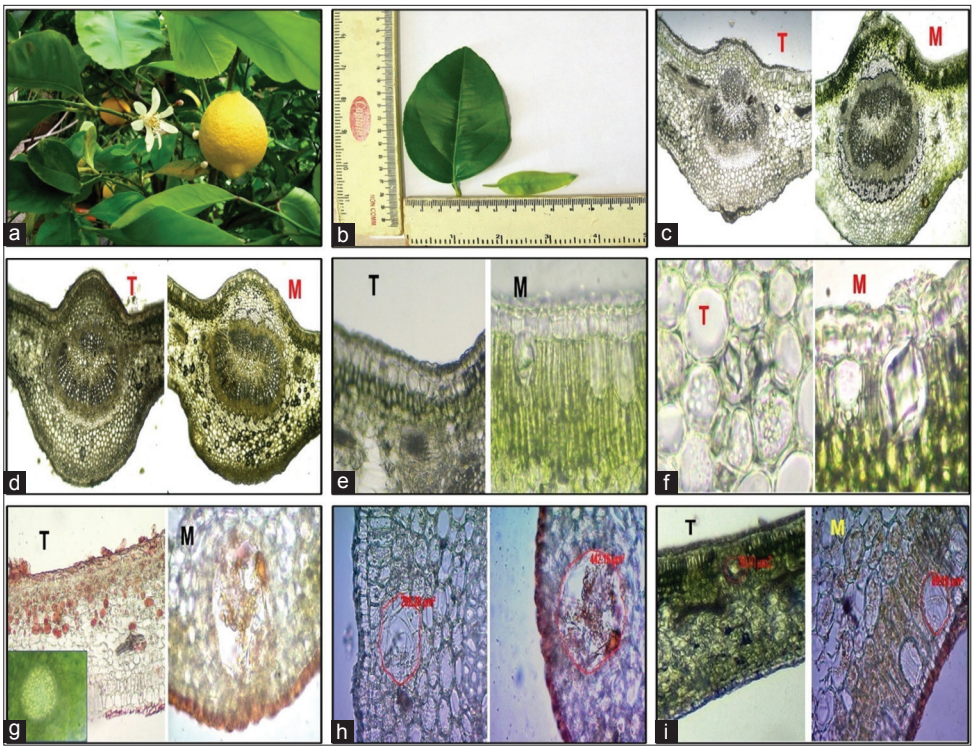

Figure 4: Morphology and microscopy of *Citrus medica* Linn. (*Bijapura*). (a) Whole plant photo, (b) Measurement of tender and mature leaf, (c) T.S. of midrib through lamina (×10), (d) T.S. after staining with $FeCl_3$ (×10), (e) Hypoderm layer (×40), (f) Crystals of calcium oxalate (×40), (g) Oil globules(×40), (h) Secretory cavity in midrib (×40), (i) Secretory cavity in lamina (×40) (T for Tender leaf; M for Mature leaf)

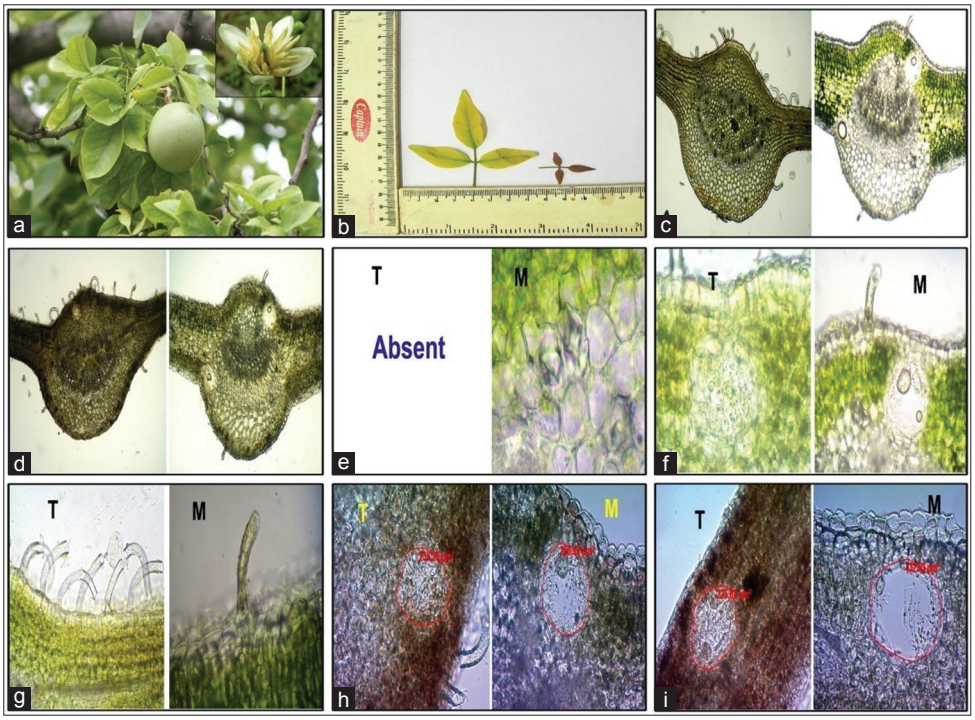

Figure 5: Morphology and microscopy of *Aegle marmelose* (L.) Correa ex Roxb (*Bilva*). (a) Whole plant photo, (b) Measurement of tender and mature leaf, (c) T.S. of midrib (×10), (d) T.S. after staining with $FeCl_3$ (×10), (e) Crystals of calcium oxalate (×40), (f) Secretory cavity containing oil globules (×40), (g) Trichomes (×10), (h) Secretory cavity in midrib (×40), (i) Secretory cavity in lamina (×40) (T for Tender leaf; M for Mature leaf)

Transverse section through midrib showed that both the epidermis consists of 6-8 layers of collenchyma followed by circularly arranged 3-5 layered pericyclic fibers. Ground tissue made up of parenchyma cells 4-5 large resinous

canals/cavities covering the vascular bundle. Ground tissue made up of parenchyma cell consists of tetrahedral prismatic crystal of calcium oxalate, tannin content and oil globules, scattered all over the tissue and inside the cavity also. Centrally, located parenchymatous cells completely surrounded by vascular bundle. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma, and its fiber [Figure 1c-i].

Mature

The diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Mesophyll tissue consists of upper compactly arranged without any air spacing 2-3 layers of the elongated palisade and lower 6-7 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 1-2 large resinous canal/cavity consists of resin, tannin, oil.

Transverse section through midrib showed that both the epidermis consists 6-8 layers of collenchyma followed by discontinuous layers of pericyclic fiber. Ground tissue made up of parenchyma cell consists prismatic crystal and interrupted by 8-10 large resin canalscovering the vascular bundle. Centrally located parenchymatous cells surrounded by vascular bundle. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber [Figure 1c-i].

S. cuminii (Linn.) Skeels

Tender

Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with thin cuticle and lower epidermis was found filled with brown content. Mesophyll tissue consists of upper compactly arranged without any air spacing 2-3 layers of the palisade and lower 8-10 layers of oval to rounded shaped compactly arranged spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina.

Transverse section through midrib showed that both the epidermis consists 6-8 layers of collenchyma followed by parenchymatous ground tissue. Collenchyma tissue interrupted by 1-2 large secretory cavities consist of crystals in few. Ground tissue made up of parenchyma cells consists cluster crystal. Arc shaped vascular bundle leads to parenchymatous ground tissue. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber [Figure 2c-i].

Mature

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Mesophyll tissue consists of upper 2-3 layers of the elongated palisade and lower 7-8 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 1-2 large secretory cavities consists of tannin, oil, brown content.

Transverse section through midrib showed that both the epidermis consists of 6-8 layers of collenchyma leads to parenchymatous ground tissue followed by 4-6 layers of pericyclic fiber. Collenchyma, in lower epidermis interrupted by 4-6 large secretory cavities. Ground tissue filled with cluster crystal, bunch of solitary crystal and rosette crystal. Vascular bundle, radially arranged and arc shaped. Outer phloem made up of phloem fibers and sieve elements and filled with tannin content, inner xylem made up of xylem vessel, xylem parenchyma [Figure 2c-i].

F. limonia (Linn.) Swingle

Tender

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells. A single layer of hypoderm was found below the upper epidermal layer. Mesophyll tissue consists of upper compactly arranged without any air spacing 4 layers of elongated palisade and lower 4-5 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 1-2 large secretory cavities consists of oil content and crystals.

Transverse section through midrib showed that both the epidermis consists of 2-3 layers of collenchyma leads to parenchymatous ground tissue. Ground tissue interrupted by 1-2 large secretory cavities. Vascular bundle circularly arranged and not well-developed. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber [Figure 3c-i].

Mature

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle consists of brown content. Single layer of hypoderm was found below the upper epidermal layer. Mesophyll tissue

consists of upper compactly arranged without any air spacing 2-3 layers of the elongated palisade and lower 6-7 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 2-3 large secretory cavities, more or less empty, consists of little amount of oil globules.

Transverse section through midrib showed that both the epidermis consists of 1-2 layers of collenchyma leads to parenchymatous ground tissue followed by 4-6 discontinuous layers of pericyclic fiber. Ground tissue consists of crystal and interrupted by 1-2 large secretory cavities. Centrally located parenchyma cell surrounded by the vascular bundle. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma, and its fiber. Vascular bundle covered with discontinuous ring of 3-4 layers of pericyclic fibers. [Figure 3c-i].

C. medica Linn

Tender

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Single layer of hypoderm was found below the upper epidermal layer. Mesophyll tissue consists of upper compactly arranged without any air spacing 1-2 layers of elongated palisade and lower 8-10 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 1-2 large secretory cavity, consists of tannin, oil.

Transverse section through midrib showed that both the epidermis consists of upper 2-3 and lower 4-6 layers of collenchyma leads to parenchymatous ground tissue. Ground tissue consists of prismatic crystals and interrupted by 1-2 large secretory cavities. Open collateral vascular bundle surrounded by discontinuous layer of pericyclic fibers. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber [Figure 4c-i].

Mature

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Single layer of hypoderm was found below the upper epidermal layer. Mesophyll tissue consists of upper compactly arranged without any air spacing 2-3 layers of elongated palisade and lower 10-12 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina along with midrib below the upper epidermal layer interrupted by an indefinite number of secretory cavity consists of tannin, oil, crystals.

Transverse section through midrib showed that both the epidermis consists of upper 2-3 and lower 5-6 layers of collenchyma leads toparenchymatous ground tissue. Centrally, located parenchyma cells surrounded by vascular bundle followed by 4-5 discontinuous layer of pericyclic fibers. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber [Figure 4c-i].

A. marmelose (L.) Correa ex Roxb

Tender

Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Mesophyll tissue consists of upper compactly arranged without any air spacing 3-4 layers of square to round shaped undifferentiated palisade and lower 5-6 layers of oval to rounded shaped without intercellular spaces spongy parenchyma cells. Between palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by 6-7 large secretory cavities consists of oil.

Transverse section through midrib showed that both the epidermis consists of 2-3 layers of collenchyma cells which leads toparenchymatous ground tissue. Collenchyma tissues toward lower epidermis contain brown content. Ground tissue consists of prismatic crystal of calcium oxalate, tannin content and interrupted by 1-2 large secretory cavities. Centrally, located parenchyma cells surrounded by open collateral vascular bundle followed by discontinuous layer of pericyclic fibers. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber. One special character, i.e. the presence of unicellular wartytrichomes were found to be crowned over the upper epidermis layer, and some trichomes were also found in lower epidermis too [Figure 5c-i].

Mature

Diagrammatic sketch showed the leaf is dorsiventral with upper palisade and lower spongy parenchyma cells. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Mesophyll tissue consists of upper 3-4 layers of elongated palisade and lower 8-10 layers of oval to rounded shaped with intercellular spaces spongy parenchyma cells. Both palisade and spongy cells were rich in chlorophyll pigments. Between palisade and spongy parenchyma vascular strands are passing

through all over the lamina. Lamina portion interrupted by maximum 1 large secretory cavity consists of crystals.

Transverse section through midrib showed that both the epidermis consists of 2-3 layers of collenchyma leads toparenchymatous ground tissue. Ground tissue consists of prismatic and cluster crystal also and interrupted by 1-2 large secretory cavities containing oils. Arc shaped vascular bundle surrounded by 3-4 discontinuous layers of pericyclic fibers. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma and its fiber. Very few (1-2) number of unicellular trichome was found to present in both upper and lower epidermis [Figure 5c-i].

All observed comparative microscopical characters of all the five leaves have been presented in a comprehensive format in Table 1.

Localization of Phyto-Constituents

M. indica Linn.*: [Figure 1d and g]*

Tender

Ground tissue and resinous canal/cavity showed the presence of orange-red color of oil globules after treating Sudan III. Pallisade cells and parenchyma cell turn bluish black when treated with $FeCl_3$ indicates the presence of tannin.

Mature

Pallisade cells and ground tissue specially phloem region turn bluish black when treated with $FeCl_3$ indicates the presence of tannin. Lamina portion as well as inside the resin canal showed the presence of orange-red color of oil globules after treating Sudan III.

Table 1: Comparative microscopical characters of tender and mature leaves of all the samples

Plant name	Characters			
	Secretory cavity/ resin canal (×4)	**Tannin content (×10)**	**Crystals (×40)**	**Oil globules (×10)**
Amra (*Mangifera indica*)				
Tender	4-5 in number in midrib 1-2 in lamina	Mostly in palisade cell and midrib	Tetrahedral prismatic crystal	Scattered all over the tissue and in cavity
Mature	8-10 in number in midrib and 1-2 in lamina	In lamina and midrib	Prismatic crystal present	Oil globules mostly in cavity and lamina
Jambu (*Syzygium cuminii*)				
Tender	1-2 in midrib	Tannin mostly filled in lamina	Cluster crystal present	Scattered all over tissues
Mature	4-6 in midrib mainly in lower surface and 1-2 in lamina	Tannin present in lamina	Cluster crystal, bunch of solitary crystal and rosette crystal	In the cavity
Kapittha (*Feronia limonia*)				
Tender	Maximum 1 in midrib and lamina	In palisade region	Crystal present in cavity	All over the tissue and in cavity
Mature	1-2 in midrib and 2-3 in lamina	In phloem region and in palisade cell	Crystal embedded into hypodermal layer	In cortical region and inside the cavity
Bijapura (*Citrus medica*)				
Tender	1-2 in midrib and maximum 1 in lamina	Present in lamina and phloem tissue	Prismatic crystal present	Oil content scattered all over and also in cavity
Mature	Many in both in midrib and lamina	In palisade cell, phloem region	Prismatic crystal in cavity	In cavity and also in lamina portion
Bilva (*Aegle marmelose*)				
Tender	6-7 in lamina and 1-2 in midrib	Tannin abundant in lamina and lower surface	Absent	In trichomes and also in all tissues
Mature	1-3 in midrib and maximum 1 in lamina	Tannin present mainly in lamina	Cluster and prismatic crystals present. Crystal also found in cavity	In cavity

S. cuminii (Linn.) Skeels: *[Figure 2d and f]*

Tender

Pallisade as well as spongy parenchyma cells and some portion ground tissue turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Orange-red color of oil globules were found scattered all over the tissues after staining with Sudan III.

Mature

Pallisade cells and phloem region turn bluish black when treated with $FeCl_3$ indicates the presence of tannin. Secretory cavity showed the presence of orange-red color of oil globules after treating Sudan III.

F. limonia (Linn.) Swingle: *[Figure 3d and f]*

Tender

Pallisade cells turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Orange-red color of oil globules were found scattered in ground tissue, lamina and also inside the secretory cavity after treating with Sudan III.

Mature

Pallisade cells turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Oil globules, were found in the cortical region and very less amount in the secretory cavity, turned orange-red color after staining with Sudan III.

C. medica Linn*: [Figure 4d and g]*

Tender

Pallisade cells as well as the secretory cavities turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Oil globules, were mainly found in the secretory cavity and also scattered all over the ground tissue, turned orange-red color after staining with Sudan III.

Mature

Pallisade cells as well as phloem region turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Oil globules were mainly found in secretory cavity and also lamina portion, turned orange-red color after staining with Sudan III.

A. marmelose (L.) Correa ex Roxb.: *[Figure 5d and f]*

Tender

Pallisade cells turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Oil globules were found in trichomes, secretory cavity and also scattered all over the ground tissue, turned orange-red color after staining with Sudan III.

Mature

Pallisade cells as well as phloem region turned bluish black when treated with $FeCl_3$ indicates the presence of tannin. Oil globules were found only inside the secretory cavity, turned orange-red color after staining with Sudan III.

Micrometric Evaluation

Resin canal is one of the characteristic features of the family Anacardiaceae as of *Amra* where secretory cavity was found in *Bilva*, *Bijapura*, *Kapittha* and *Jambu*, is an important diagnostical tool of the family Rutaceae and Myrtaceaea. The measurement of the resin canal and secretory cavities were taken and described properly in Table 2 [Figures 1-5h and i].

Surface Study

Detail surface study, i.e., type of stomata, stomatal length and breadth, stomatal index, vein-islet number were evaluated and given in Table 3 [Figures 6-8d].

Powder Microscopy

Diagnostic powder characters of the tender and mature leaf of all five plants (*Amra*, *Jambu*, *Kapittha*, *Bijapura* and *Bilva*) has been described properly in Table 4 [Figures 6-8a-c].

DISCUSSION

The present study was conducted to demonstrate the similar and different characters of tender and mature leaves of these five plants under *Panchapallava* group regarding its phyto cellular constituents along with morphological detail.

Table 2: Micro measurements of secretory cavities/ resin canals

Plant name	Characters	
	Resin canal (midrib) (×40)	**Resin canal (lamina) (×40)**
Amra (*Mangifera indica*)		
Tender	489.81 μm²	203.08 μm²
Mature	609.16 μm²	211.93 μm²
	Secretory cavity (midrib) (×40)	**Secretory cavity (lamina) (×40)**
Jambu (*Syzygium cuminii*)		
Tender	396.71 μm²	Absent
Mature	332.13 μm²	207.75 μm²
Kapittha (*Feronia limonia*)		
Tender	677.51 μm²	284.85 μm²
Mature	229.09 μm²	1155.75 μm²
Bijapura (*Citrus medica*)		
Tender	202.28 μm²	30.01 μm²
Mature	442.16 μm²	89.19 μm²
Bilva (*Aegle marmelose*)		
Tender	235.25 μm²	225.02 μm²
Mature	256.97 μm²	432.63 μm²

Table 3: Detail surface study of *Panchapallava* leaves

Plant name	Type of stomata	Length and width of stomata in mm (×40)		Stomatal index in Sq. mm (×40)		Vein-islet number in Sq. mm (×10)
		U.E	L.E	U.E	L.E	
Amra (*Mangifera indica*)						
Tender	Paracytic	Nil	L-0.04; W-0.04	Nil	18.16	4-6
Mature	Paracytic	Nil	L-0.085; W-0.08	Nil	16.24	5-7
Jambu (*Syzygium cuminii*)						
Tender	Paracytic	Nil	L-0.1; W-0.105	Nil	20	3-5
Mature	Paracytic	Nil	L-0.1; W-0.12	Nil	19.32	4-5
Kapittha (*Feronia limonia*)						
Tender	Actinocytic	L-0.1; W-0.075	L-0.04; W-0.045	5.039	12.174	2-3
Mature	Actinocytic	L-0.1; W-0.093	L-0.085; W-0.08	4.569	10.234	3-5
Bijapura (*Citrus medica*)						
Tender	Anomocytic	Nil	L-0.07; W-0.075	Nil	13.858	3-5
Mature	Anomocytic	Nil	L-0.115; W-0.115	Nil	13.783	7-9
Bilva (*Aegle marmelose*)						
Tender	Anomocytic	L-0.1; W-0.093	L-0.085; W-0.085	15.326	16.66	2-4
Mature	Anomocytic	L-0.1; W-0.1	L-0.115; W-0.1	9.545	16.304	3-5

mm: millimeter; U.E: Upper epidermis, L.E: Lower epidermis

Table 4: Powder microscopical characters of all *Panchapallava* plants

Plant name	Microscopical powder characters (×40)
Amra (*Mangifera indica*)	
Tender	Cluster, prismatic crystal, wavy parenchyma cell, oil globule, fragment of epidermal cells, fragment of palisade cell
Mature	Cluster, prismatic crystal, parenchyma cell, oil globule, fiber, pitted vessel, group of sclereids
Jambu (*Syzygium cuminii*)	
Tender	Unicellular warty trichome, fragment of palisade cells, prismatic crystal, oil globule, fiber
Mature	Prismatic crystal, brown content, oil globule, fibers, annular vessel
Kapittha (*Feronia limonia*)	
Tender	Oil globule, prismatic crystal, trichome, brown content, fiber, sclereid
Mature	Oil globule, prismatic crystal, trichome, fiber, fragment of palisade cell
Bijapura (*Citrus medica*)	
Tender	Prismatic crystal, brown content, fragment of annular vessel, trichome, fragment of epidermal cell
Mature	Group of prismatic crystal, pitted vessel, trichome, group of fibers, pitted fiber
Bilva (*Aegle marmelose*)	
Tender	Ample amount of warty trichomes, prismatic crystal, oil globule, fragment of palisade cells, fragment of annular vessel
Mature	Trichomes less than tender leaf, crystal, oil globule, fragment of epidermal cells, fiber.

Result showed that Rutaceae, Anacardiaceae and Myrtaceae family plants are dominantly tree or shrub. Tender and mature leaves of all individual plants (*Amra, Jambu, Kapittha, Bijapura,* and *Bilva*) showed similar morphological characters except the length and width of individual.

Microscopical study of all individual plants (both tender and mature) showed some characteristic features which justify the relevant family characters of that plant. The presence of resin canal and prismatic crystal in *Amra* reflect the character of the family Anacardiaceae whereas, presence of secretory cavity in *Kapittha*, *Bijapura* and *Bilva*, layer of hypoderm

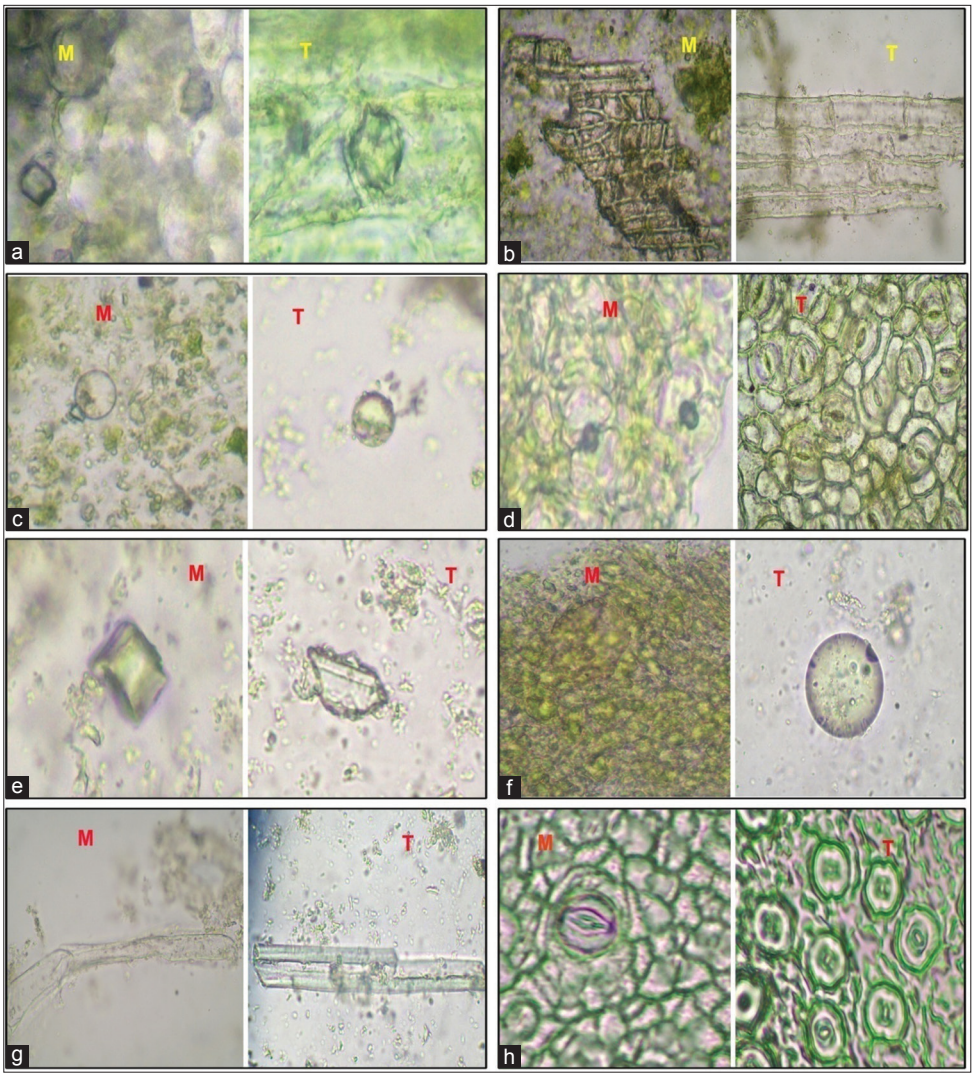

Figure 6: Powder microscopy of *Amra* and *Jambu,* (a) Crystals of *Amra* leaf (×40), (b) Wavy parenchyma cells in *Amra* leaf (×40), (c) Oil globule in *Amra*lea f(×40), (d) Stomata of *Amra*leaf (×40), (e) Crystal in *Jambu*leaf (×40), (f) Oil globules in *Jambu*leaf (×40), (g) Fiber in *Jambu*leaf (×10), (h) Stomata in *Jambu*leaf (×40) (T for Tender leaf; M for Mature leaf)

in *Bijapura* and *Kapittha* and trichomes in *Bilva* justify the family character of Rutaceae. *Jambu* showed the presence of secretory cavity and cluster crystals which make it compatible with the family Myrtaceae.

Transverse sections of both tender and mature leaves of *Amra* were found same except the layer of pericyclic fibers, which were found 3-5 layers in case of tender leaf and discontinuous layers in case of mature leaf. Some similar characters like presence of tannin, prismatic crystals, etc. were found in both tender and mature leaves. Transverse section of mature and tender leaves showed the difference in palisade layer like tender leaf showed compactly arranged palisade layer whereas mature leaf showed elongated palisade layers. Transverse section of mature and tender leaves of *Kapittha* showed similar structure along with one typical character, i.e., the presence of single layered hypoderm cell below the upper epidermal layer. In case of tender leaf, vascular bundle was found under developed in compare to mature leaf. Transverse section of tender leaf of *Bijapura* showed under developed pericyclic layers in compare to mature leaf. One typical character was found in mature leaf like lamina along with midrib below the upper epidermal layer interrupted by indefinite number of secretory cavity consists of crystals, oils. Transverse section of *Bilva* tender leaves showed undifferentiated square to round shaped palisade layer whereas mature leaves showed well defined elongated palisade cell, may be due to the maturity of the leaf along with its cell content. One distinctive character, i.e. the presence of unicellular warty trichomes was observed in both tender and mature leaves. It was found that tender leaf contains indefinite number of trichomes whereas mature leaf showed only 1-2 numbers of trichomes. Trichomes are mainly reported to control the rate of transpiration as well as to reduce heating effect of sunlight and to protect the plant body from outer injurious parts, which is more needful for young plant than the mature.[20]

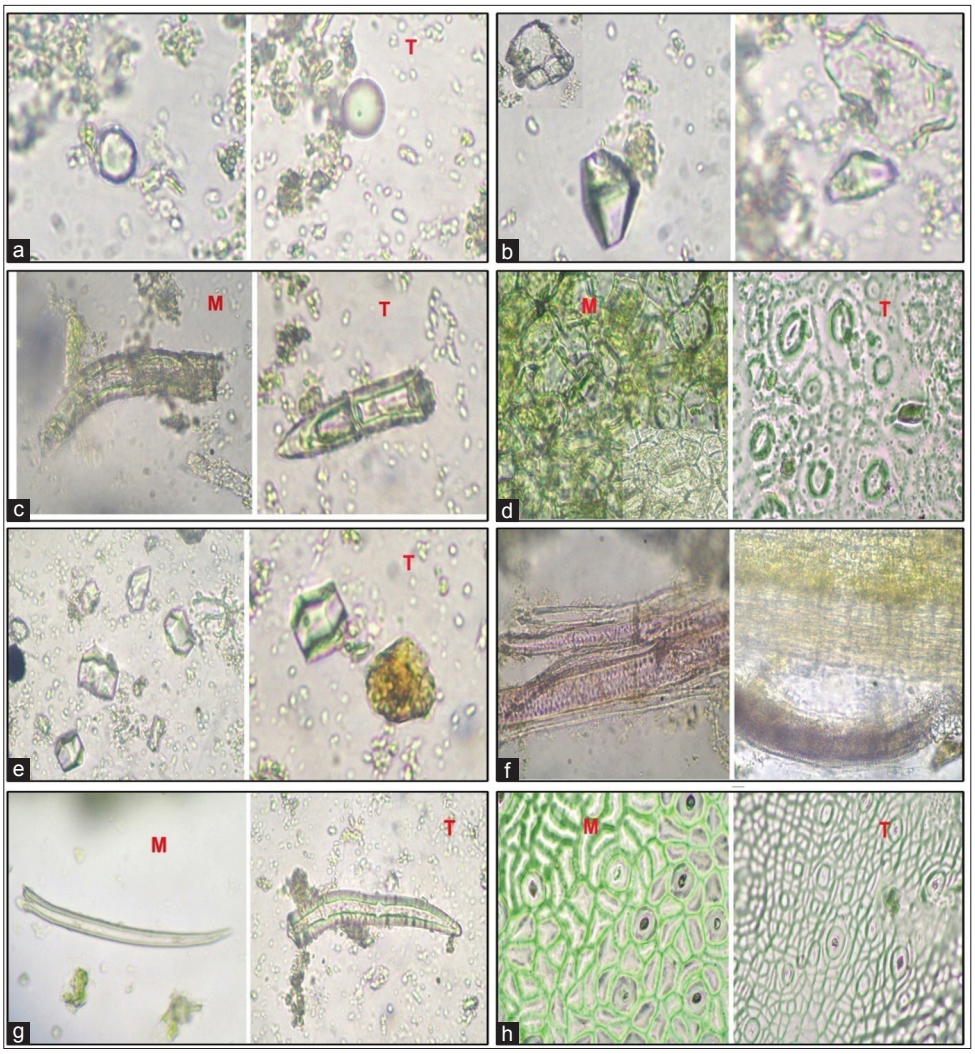

Figure 7: Powder microscopy of *Kapittha* and *Bijapura,* (a) Oil globule in *Kapittha* leaf (×40), (b) Crystal in *Kapittha* leaf (×40), (c) Trichome in *Kapittha* leaf (×10), (d) Stomata in *Kapittha*leaf(×40), (e) Crystals in *Bijapura* leaf (×40), (f) Pitted vessel and annular vessel of *Bijapura*leaf(×40), (g) Trichome in *Bijapura* leaf (×10), (h) Stomata in *Bijapura* leaf (×40) (T for Tender leaf, M for Mature leaf)

Localization and micro measurement showed that resin canals were found in both tender and mature leaves of *Amra* but they differ in number, size and its content. The size of the canal in matured leaf was found larger in diameter than the tender leaf. Same things were found in case of rest of the plants, i.e., secretory cavities were found in both tender and mature leaves of rest of four plants (*Jambu*, *Kapittha*, *Bijapura,* and *Bilva*). In lamina portion, diameter of cavity was found large in the case of mature leaf than the tender wherein midrib it varies like cavity in tender leaf of *Jambu* and *Kapittha* was found large in diameter than mature. A number of cavities was found to be more in mature leaf than tender except *Bilva*. Beside all these, oil globules all over the tissues and also in cavities/canals were found more in the case of tender leaves than the mature leaves and it was also found that cavities become empty in case of mature leaves, may be due to the utilization of oil content with the maturity of the leaf.

Surface study showed that the stomatal index in all five tender leaves were more than that of mature leaf, whereas the size of stomata was found much bigger in mature leaves than the tender leaves which indicate the presence of smaller but more number of stomata in tender leaves than the mature leaves because of proliferation of meristematic tissue growth and development in the young parts of plant, signifies or justifies the presence of more number of stomata in tender leaves than mature leaves.[21]

Most of the powder characters were found similar in the case of tender and mature leaf of all individual plants (*Amra, Jambu, Kapittha, Bijapura,* and *Bilva*). Beside these similar characters some different characters were also found which normally represent the quality of tender and mature leaf. The presence of some characters such as of fiber, pitted vessel, group of sclereids, specifies the maturity of leaves of *Amra.* Powder microscopy of *Jambu* showed presence of unicellular warty trichomes only in tender leaves whereas *Bilva, kapittha* and *Bijapura* showed the presence ample amount of trichomes in tender leaves than the mature leaves, which represent the normal character of trichomes which is found to be present

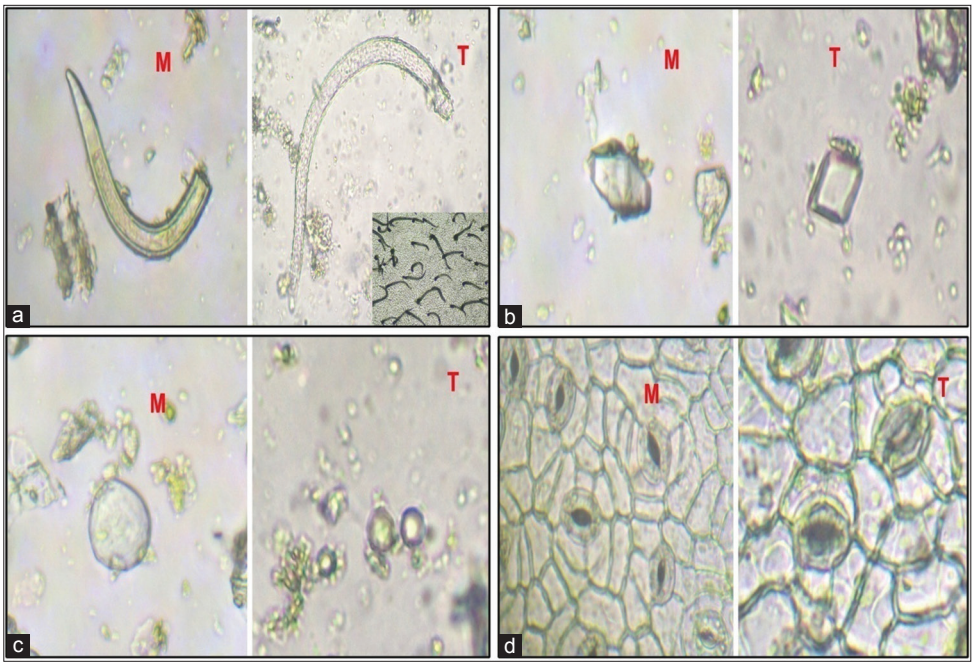

Figure 8: Powder microscopy of *Bilva*. (a) Trichome in *Bilva* leaf (×40), (b) Crystals in *Bilva* leaf (×40), (c) Oil globule in *Bilva* leaf (×40), (d) Stomata in *Bilva* leaf (×40) (**T** for Tender leaf; **M** for Mature leaf)

in more amount in young condition than the maturity because previously it already has been described that trichomes are mainly reported to control the rate of transpiration as well as to reduce heating effect of sunlight and to protect the plant body from outer injurious parts, which is more needful for young plant than the mature.[21]

Powder character of *Bijapura* also showed the presence of the annular vessel in tender leaves and pitted vessel in mature leaves which signifies the development stages of the vessel with maturity.

CONCLUSION

The present study concludes that there is no much difference in the anatomical structure of the tender and mature leaves of plants included under *Panchapallava* except the size of the secretary cavity and resin canal, in the case of most of the *Panchapallava* plants; it is less in tender and comparatively bigger in mature leaves. The tender leaves contain more oil globules which are not found in the mature leaves. The observation of the present study may provide a clue to take further research work to evaluate the detail analytical and pharmacological properties of the tender and mature leaves of *Panchapallava*.

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