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Research Article

DETAILED COMPARATIVE PHARMACOGNOSTICAL STUDY OF *ANNONA SQUAMOSA* LINN. AND *ANNONA RETICULATA* LINN. LEAVES

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ABSTRACT:

Annona squamosa Linn. and Annona reticulata Linn. are locally known as Sitaphala and Ramphala respectively both belongs to family Annonaceae. Leaves of both are used in various diseases like suppurant, toothache, anthelmintic commonly. Individually *A. squamosa* is used in anti diabetic, antispasmodic, dandruff and *A. reticulate* is used in flatulence and toothache. Till date there is no scientific comparative study has been reported. Pharmacognostical study of *A. squamosa* shows lysogenous cavity and sparse trichome where as, *A. reticulate* shows multicellular trichomes filled with tannin and stone cells. The powder characters of *A. squamosa* are stone cells and prismatic crystals of calcium oxalate whereas *A. reticulate* shows pitted stones cells and micro rosettes crystals of calcium oxalate. Annular vessels, lysogenous cavity and paracytic stomata are common characters observed in both the leaves. **KEY WORDS:** Annona squamosa, Annona reticulata, Annonaceae, Leaf.

INTRODUCTION:

Annona squamosa Linn. and Annona reticulata Linn. belongs to family Annonaceae. A.squamosa vernacular name is "sharifa" or "sitaphala". It is a small tree with erect woody and branched stem; leaves are simple, alternate, oblanceolate, entire and obtuse¹. The fruits are edible and English name is custard apple, apart from this the leaves are reported to possess stimulant, antispasmodic, anthelmintic & insecticidal properties. The crushed leaves are reported to be applied to the nostrils in hysteria and fits. The poultice of leaves is used as a cataplasm over boils and ulcers to induce suppuration. It relieves pains and swellings. Moreover Ethnobotanical studies reveals that the leaves are used in ulcers, in scabies, boils, for toothache, wounds, cuts, dysentery, sprains, dandruff, to remove lice,, in diarrhoea, anti diabetic².

A.reitculata vernacularly known as "Ramphala" is Small deciduous tree. Leaves acuminate, 5-8 in. long, green beneath, nerves divergent. Flowers 2-3 together; innermost tepals narrow-oblong, pale green. Fruits are orange, subglobose, with flat 5-cornered areoles. Tree becomes leafless when fruits mature ³. Apart from edible fruit, the leaves of this plant are used as anthelmintic & externally as suppurant. Ethnobotanical studies reveal that the leaves are found to be useful in flatulence and toothache ⁴. Till date there is no scientific comparative study has been reported, hence this study was undertaken. The main objective is to study the transverse sections, powder microscopy including micrometry evaluation.

MATERIALS & METHODS:

Collection:

The fresh leaves were collected from the Botanical Garden I.P.G.T. & R.A. Noted Annona *squamosa* as A and *Annona reticulata* as B.

Macroscopic evaluation 5,6

Macroscopic characters of both the leaves were recorded as per visual observation.

Organoleptic evaluation 5,6

The colour, odour, and taste of both the leaves were recorded separately.

Microscopic evaluation 5,6

Free hand sections were taken, cleared with chloral hydrate and then with phloroglucinol and hydrochloric acid. Histochemical tests for constituents like tannin, mucilage etc. was done. Microphotographs were taken by using Carl Zeiss binocular microscope attached with camera.

Surface study^{5, 6}

Leaf surfaces are studied by scraping and also peeling up of both the surfaces of both leaves, then washed with chloral hydrate and observed under microscope for stomatal structure and distribution and epidermal cells.

Micrometry^{5, 6}

Systematic evaluation of Pharmacognosy of leaf study followed by micrometry, Carl Zeiss binocular microscope attached with camera with preloaded micrometric analysis software, measure the length, breath of the leaf characters like stomata, trichomes etc. mean value is taken into consideration.

Powder microscopy⁷

Cut pieces of leaf were dried under shade, powdered with help of mechanical grinder and sieved through mesh no.60. Leaf powder was studied under microscope with distil water, stained with phloroglucinol and hydrochloric acid.

Histochemical tests 8

To detect the site of location of various constituents of the drug, sections of both leaves were treated with various reagents like Sudan III (for Oil), FeCl3 to (tannin) and iodine for (starch grains) etc.

RESULTS AND DISCUSSION: MORPHOLOGY

A. squamosa (A)

Leaves simple, alternate to spirally arranged on the zigzag twig, petiolate, petiole measures about 1-1.5 cm, twisted and channeled, stipulate, stipule early withering, measuring about 0.2-0.5 mm ,stipules linear, , leaf ovate to lanceolate, margin simple, lamina measures about 10x5 cm, lamina base simple, dark green above light green below, midrib strong at lower surface lateral veins 10-11 and vein lets tended to meet

margin of the leaf, leaf tip obtuse, upper surface glabrous, lower surface glocus, offensive smell. (Photo A1).

A.reticulata (B)

Leaves simple, alternate, petiolate, petiole measures about 1-1.6 cm, slightly twisted and channeled, stipulate, stipule early withering, measuring about 0.3-0.6 mm ,stipules linear, , leaf oblanceoalate, margin simple, lamina measures about 15x8 cm, lamina base simple, dark green above light green below, , midrib strong at lower surface lateral veins 15-17 and vein lets tended to meet margin of the leaf, leaf tip acute, light green in colour on both the sides, less pungent and very thin as compared to *A. squamosa*. (Photo B1).

MICROSCOPY

T.S. of Petiole A

Petiole twisted and measures about 1-1.5 cm, T.S. of the petiole shows slightly cordate in outline. Outermost layer consist of irregular shaped single layered epidermal cells with thick cuticle followed by parenchymatous cortex, consist chlorophyll pigments, oil globules and micro rosette crystal of calcium oxalate spread all over the cortex. Randomly distributed group of stone cells circularly arranged at the region of cortex. Vascular bundles (5-6 groups) raidally arranged, metaxylem towards periphery and protoxylem towards pith, phloem situated above the xylem with some sieve elements. Vascular bundles group above the phloem covered by thick walled, lignified 3-5 layers of pericyclic fibers. Pith occupied by group of stone cells. (Photo A2, A3)

T.S. of Petiole B

Petiole slightly twisted and measures about 1-1.6 cm, T.S. of the petiole shows deeply orbicular in outline. Outermost layer consist of irregular shaped single layered epidermal cells with cuticle, having multi cellular trichomes filled with tannin content, followed by parenchymatous cortex, consist chlorophyll pigments, oil globules and micro rosette crystal of calcium oxalate spread all over the cortex. Randomly distributed 7-8 group of stone cells circularly arranged at the region of cortex. Vascular bundles (7-8 groups) raidally arranged metaxylem towards periphery and protoxylem towards pith, phloem situated above the xylem. Pith occupied by parenchymatous cells, central most part of the pith occupied by group of stone cells. Stone cells are lignified, pitted and having wide lumen. (Photo B2, B3, B4)

T.S. of leaf A

Transverse section through midrib shows the upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and rarely simple trichomes on lower surfaces. Lamina upper 1-2 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma throughout the lamina lysogenous cavities are very common, prismatic crystals , oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially arranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells. (Photo A4, A5, A6)

T.S. of leaf B

Transverse section through midrib shows the upper and lower single layered compactly arranged rectangular to barrel shaped epidermis with thick cuticle and multicellular trichomes filled with tannin on lower surfaces. Lamina upper single layered palisade parenchyma and lowers 6-7 layers of spongy parenchyma lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially arranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells, in center a group of stone cells in observed. (Photo B5, B6, B7, B8)

SURFACE STUDY

Both the leaves show the upper and lower surfaces peeled out and observed under the microscope, the upper epidermis shows only epidermal cells and lysogenous cavity and oil globules whereas lower epidermis shows paracytic stomata epidermis cells, lysogenous cavity, oil globules (Photo A7, A8 and B9, B10)

MICROMETRY

Both the powder characters were scientifically measured i.e. length x breath, circumference of stomata, fiber, prismatic crystal, trichomes etc. mean values are taken into consideration. Results are given in Table no. 1.

POWDER MICROSCOPY

Diagnostic character of sample A powder shows paracytic stomata from lower surface, fragment of fibres with narrow lumen, multicellular trichome filled with tannin content from epidermal surface, prismatic crystal of calcium oxalate, fragments of palisade parenchyma from upper epidermis, stone cells, spiral and annular vessels from vascular bundle. (Photo A9-A15)

Diagnostic character of sample B powder shows paracytic stomata from lower surface, fragment of fibres with narrow lumen, multicellular trichome filled with tannin content from epidermal surface, micro rosette crystal of calcium oxalate, pitted stone cells with wide lumen, annular vessels from vascular bundle (Photo B11-B18).

HISTOCHEMICAL TESTS

To detect the site of location of various constituents of the drug, sections of both leaves were treated with various reagents; the results are depicted in Table No. 2.

CONCLUSION

From the results it can be concluded that both the leaves show some similar and also distinguishing characters in morphology, microscopy, and also in micrometric studies. These characters play an important role in identification of specific species even in powder form. The study was completed with minimum qualitative standards as prescribed by API at preliminary level. The results of this study may be used as the reference standard in further research undertakings of its kind.

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Characters	Α	В	
Prismatic crystals of calcium	25.81μm ² 226.5 μm ²		
oxalate			
Oil globules	-	33.365 μm ²	
Fiber	94.57x5.76 μm	150.5x2.71 μm	
Stomata	9.5x5.0 μm	8.3x3.86 μm	
Stomata circumference	45.50 μm ²	57.5 μm ²	
Trichome	40.5x4.07 µm	38.03x3.90 µm	
Epidermal cells	36.50 μm ²	37.55 μm ²	
Stone cells	250.50 μm ²	340.97 μm ²	
Annular vessel	+43.7x5.83 μm	+45.50x5.50 μm	

Table No. 2 Histochemical tests							
Sr. No.	Reagents	Observation	Characteristics	A	В		
1.	Phloroglucinol+Conc. Hcl	Red	Lignified cells	++	++		
2.	Iodine	Blue	Starch grains				
3.	Phloroglucinol+Conc. Hcl	Dissolved	Calcium oxalate crystals	++	++		
4.	FeCl ₃ solution	Dark blue to black	Tannin cells	++	++		
5.	Sudan III	Red	Oil	++	++		

++ present. - Absent



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BIS group of pitted stone tens



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