Int J Pharma Res Health Sci. 2018; 6 (2): 2540-45
DOI:10.21276/ijprhs.2018. 2018. 02.35
Fichadiya G *et al*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Morphological, Structural & Micrometric Evaluation of Cystoliths & Trichomes of Cucurbitaceae family w.s.r. to *Cucumis prophetarum* L.

Gautam Fichadiya [1,*], Harisha C R [2], Switu Jani [3]
*[1]Ph. D. Scholar, Pharmacognosy Lab., IPGT & RA, GAU, Jamnagar, India.
[2] Head, Pharmacognosy Lab., IPGT & RA, GAU, Jamnagar, India.
[3] SRF, Dept. of Dravyaguna, IPGT & RA, GAU, Jamnagar, India.

ARTICLE INFO

Received: 16 Apr 2018
Accepted: 27 Apr 2018

ABSTRACT

Objective: Species and genera can also be recognized on the basis of structure of trichome and on the types of cystoliths. Smaller variations in size and surface area should be treated as a basis for the separation of closely related genera and species only after scientific investigation. The present study is undertaken with 6 different genus of the family Cucurbitaceae to establish the micro-morphological variations of trichomes and cystolith.
Experimental approach: Collection of the sample plants, their morphology, microscopy and micrometry were done as per the standard procedure.
Findings: *C. prophetarum* leaf shows triangular to irregular cystoliths (0.32-3.66µm²) in epidermal region, while in *M. charantia* balloon shaped cystoliths (1.04-2.14µm²) were observed in the surface study. Sessile glandular trichomes presents in *C. prophetarum* which is absent in *C. grandis*.
Discussion: there were total 16 types of trichomes observed here from which 10 types of trichomes were non glandular while 6 types of trichomes were glandular. The longest multicellular trichome is present in *M. charantia* measuring about 10.53-15.04 × 0.98-1.18 µm. Irregularly shaped cystoliths are found in all the plant samples expect where *C.prophetarum* bears biggest size of cystolith.
Conclusion: It can be conclude that cystoliths and trichomes can help to differentiate the genus belonging to family *Cucurbitaceae* by their types, size and shape at microscopic level.

Keywords: *Cucumis prophetarum*, Cucurbitaceae, Cystoliths, Pharmacognosy, Trichomes

1. INTRODUCTION

Cucurbitaceae family includes 110 genera and between 650 to 850 species, distributed mainly in tropical and subtropical regions of the world. Its 37 genera and about 100 species have been reported from India. Some of the important medicinal genuses of the family are *Momordica, cucumis, Coccinia, Luffa, Lagenaria* and *Cucurbita*.[1]

Corresponding author *
Gautam Fichadiya
Ph. D. Scholar, Pharmacognosy Lab., IPGT & RA, GAU, Jamnagar, India
E mail: fichadiyagautam@gmail.com

Cucurbitaceae family embraces the characters of possessing glandular and non glandular types of trichomes along with varieties of cystoliths.[2] An elongated tubular outgrowth of an epidermal cell is termed as trichome or hair. Trichome consists of two parts viz. root and body. They are present in most of the parts of the plant. Depending upon the structure and the number of cells present in trichomes, they are classified in various types such as covering, glandular and hydathodes types of trichomes.[3] Trichomes types have been successfully used in classification of genera and even of species in certain families and in the recognition of inter-specific hybrids.[4] A particular type of hair is often characteristic of the plant family or genus.[5]

Crystals are non-nitrogenous waste products or excretion of plants. They may be present in cell wall or cell cavity. Crystals are produced in an intriguing variety of shapes and its morphology depends on the taxonomic group of the plants. The common forms of crystals consist of calcium oxalate, calcium carbonate and silica.[6] Calcium carbonates or cystoliths which are mineral concretion in the cells of leaves,[7] rarely occur in well formed crystalline structure. They are restricted to only 15 families such as Acanthaceae, Boraginaceae, Begoniaceae, Campanulaceae, Cannabinaceae, Cistaceae, Flacourtiaceae, Hernandiaceae, Loasaceae, Opiliaceae, Scrophulariaceae, Ulmaceae, Cucurbitaceae, Moraceae and Urticaceae.[8]

Cystolith is a spindle-shaped body composed of concentric layers of longitudinally oriented cellulose micro fibrils associated with pectin and other cell wall polysaccharides. At maturity it is heavily impregnated with calcium carbonate. Some cystoliths also contain silicon and are covered in a sheath of siliceous material. Cystolith formation occurs at the tip of a peg that grows in from the lithocyst wall.[9] They may be single or double cystolith, grape shaped, cigar shaped, balloon shaped or round.[10]

Review revealed that trichomes and cystoliths have successfully proved to differentiate species among same families,[11-13] curcurbitaceae being a family of medicinal importance, is not yet explored in the light of trichomes and cystoliths for differentiation among various genus. Hence the present study is done to differentiate the well known Ayurvedic drugs of family Cucurbitaceae with reference to cystoliths and trichomes with critical observation and micro-measurements of them on plants *Coccinia grandis* L., *Cucumis prophetarum* L., *Luffa cylindrica* L., *Momordica charantia* L., *Lagenaria leucantha* Duch., *Luffa acutangula* L.

2. MATERIALS AND METHODS

Collection:

The fresh matured female plant twigs of *Coccinia grandis, Cucumis prophetarum, Luffa cylindrica, Momordica charantia, Lagenaria leucantha, Luffa acutangula* were collected from the natural habitat of the Jamnagar forest area as per collection standards.[14]

Morphology:

Leaves characters such as shape, size, base, margin, apex and phyllotaxy are scientifically studied as per taxonomic system.[15]

Histochemical tests for cyctoliths:

Sections of the plant samples were subjected to histochemical tests to identify the cystoliths and its reaction by treating with various chemical reagents.[6]

Microscopic evaluation:

To observe trichomes, cystoliths and their distribution thin free hand transverse sections of leaf passing through midrib and petiole of all six plants were taken and were observed in distilled water under the microscope. Microphotographs were taken by using Carl Zeiss binocular microscope attached with camera.[16]

Surface study:

For the observation of cystolyths, epidermis was exposed by scrapping of the other tissues. Wet leaf was placed on glass slide and tissues were scrap off with sharp edge of blade carefully. Water was slowly added and scrapping was done until transparent epidermis was obtained.[17]

Micrometry:

Systematic evaluation of trichomes and cystolith study followed by micrometry as it is one of the differentiating tool; Carl Zeiss trinocular microscope attached with camera with preloaded micrometric analysis software, measure the length and breadth of the trichomes and some cystolith, area of some trichomes and cystolith, mean value is taken into consideration.[18]

3. RESULTS AND DISCUSSION

Identification and chemical reaction of cystoliths

The identification and its reaction with various reagents are depicted in the table no. 1.

***Coccinia grandis* L.**

Morphology: Leaves are simple, alternate, petiolate, broadly ovate or ovate triangular, entire or 3 angled deeply lobbed, glabrous, base cordate. Obstuse apex, Upper surface dark green while lower surface light green and measures about 3.1-6.6 cm × 2.9-5.7 cm. Petiole slender, 1.2 cm to 2.5 cm long, light green in colour. [Figure 1]

TS of leaf: Transverse section of *C. grandis* leaf passing through midrib shows simple multicellular trichomes while TS of petiole shows unicellular warty trichome. [Figure 1] The micro-measurement of the trichomes are given in table no. 2.

Surface study: Lower surface study of *C. grandis* leaf shows irregular shaped calcium carbonate deposition with various size. [Figure 1] The micro-measurement of the cystolith are given in table no. 3.

***Luffa cylindrica* L.**

Morphology: Leaves are broadly ovate, glabrous, petiolate, Upper surface green in color with brownish tinge, measures about 5.1-9.3 cm × 7.5-11.8 cm. Petiole measures about 2.4 cm to 3.6 cm long. [Figure 1]

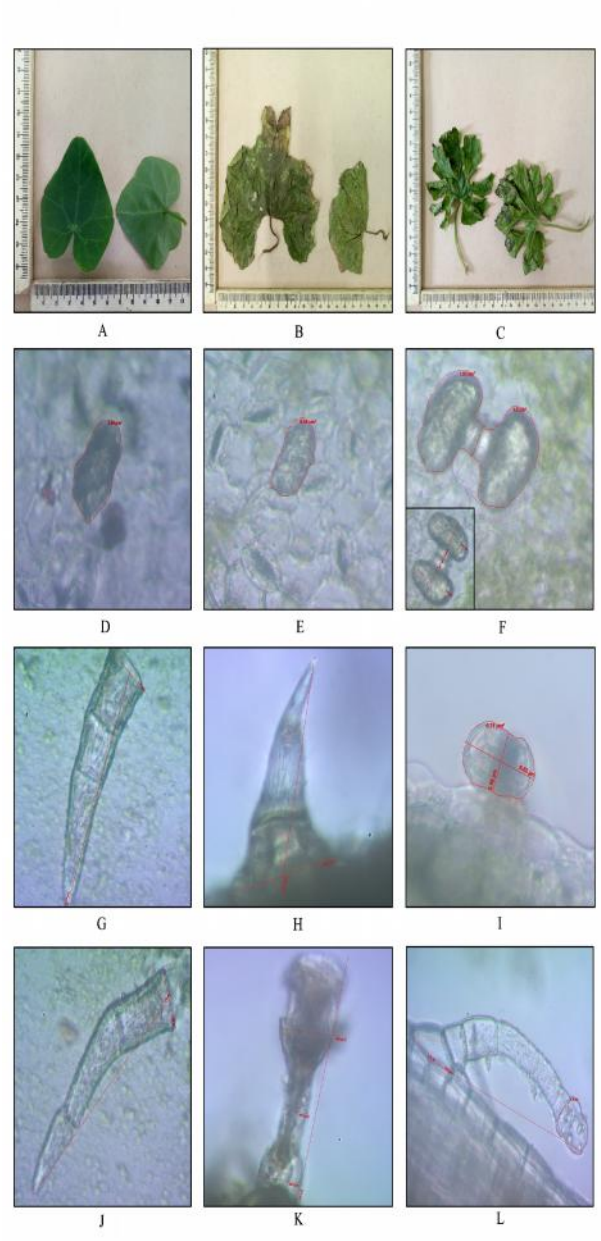

Fig 1: Trichomes and cystolith of *C. grandis, L.cylindrica, M. charantia*
A - Leaf macroscopy of *Coccinia grandis*
B - Leaf macroscopy of *Luffa cylindrica*
C - Leaf macroscopy of *Momordica charantia*
D - Micromeasurement of cystolith of *C. grandis*
E - Micromeasurement of cystolith of *L. cylindrica*
F - Micromeasurement of cystolith of *M. charantia*
G - Micromeasuremets of multicellular trichome of *C. grandis*
H - Micromeasuremets of multiseriate warty covering trichome of *L. cylindrica*
I - Micromeasuremets of sessile glandular trichome of *M. charantia*
J - Micromeasuremets of multicellular warty trichome of *C. grandis*
K - Micromeasuremets of multicellular trichome with shrunken cell of *L. cylindrica*
L - Micromeasuremets of multicellular stalk with head of *M. charantia*

TS of leaf: Transverse section of *L. cylindrica* leaf passing through midrib shows multicellular warty trichome, multiseriate multicellular covering trichome, multicellular stalk with glandular head, calcified multiseriate multicellular warty trichome while TS of petiole shows multicellular stalk with glandular head trichome. Warty multicellular covering trichome was present in TS of midrib as well as petiole. [Figure 1] The micro-measurement of the trichomes are given in table no. 2.

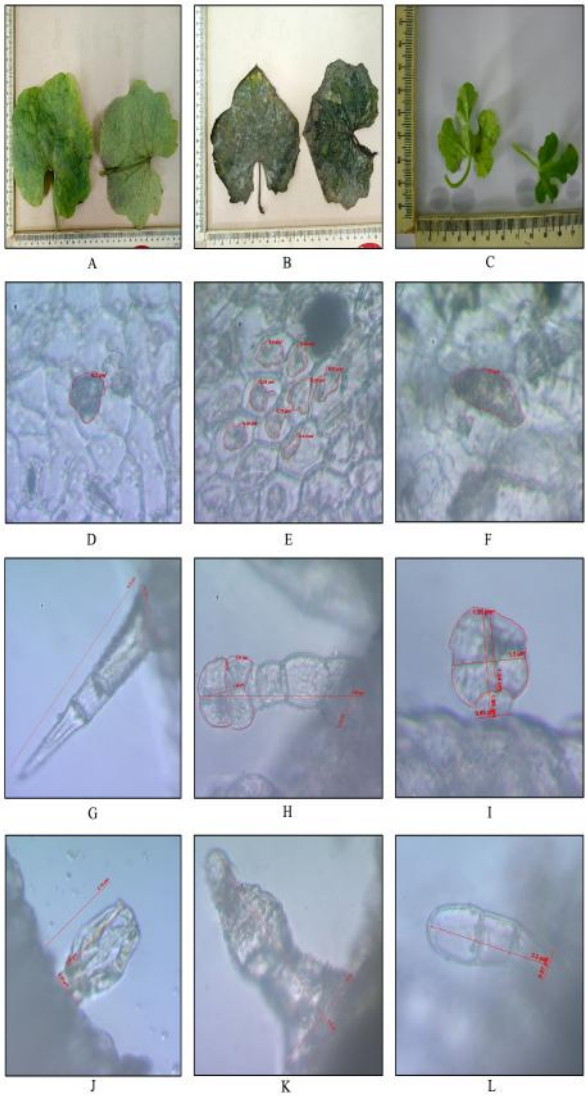

Fig 2: Trichomes and cystolith of *L.leucantha, L. acutangula, C. prophetarum*
A - Leaf macroscopy of *Lagenaria leucantha*
B - Leaf macroscopy of *Luffa acutangula*
C - Leaf macroscopy of *Cucumis prophetarum*
D - Micromeasurement of cystolith of *L.leucantha*
E - Micromeasurement of cystolith of *L. acutangula*
F - Micromeasurement of cystolith of *C. prophetarum*
G - Micromeasuremets of multicellular covering warty trichome of *L.leucantha*
H - Micromeasuremets of multicellular stalk and head trichome of *L. acutangula*
I - Micromeasuremets of multiseriate stalk multicellular head trichome of *C. prophetarum*
J - Micromeasuremets of unicellular stalk two celled head trichome of *L.leucantha*
K - Micromeasuremets of glandular shaggy trichome of *L. acutangula*
L - Micromeasuremets of pleuricellular trichome of *C. prophetarum*

Surface study: Lower surface study of *L. cylindrica* leaf shows irregular shaped calcium carbonate deposition with various size. [Figure 1] The micro-measurement of the cystoliths are given in table no. 3.

***Momordica charantia* L**

Morphology: Leaves are deeply 5-9 lobed, membranous, glabrous, petiolate, upper surface dark green in colour and lower surface light green in colour with prominent 5-7 ribs, covered with hairs and measured about 4.2-11.5 cm × 4.1-7.1 cm. Petiole long, slender and pubescent measured about 3.1 cm to 6.2 cm long. [Figure 1]

TS of leaf: Transverse section of *M. charantia* leaf passing through midrib shows multicellular trichome and glandular hairs with multicellular stalks while TS of petiole shows multicellular warty trichome, sessile glandular and calcified multicellular trichome. multicellular stalk with multicellular head and multicellular trichome with shrunken cell were present in TS of midrib as well as petiole. [Figure 1] The micro-measurement of the trichomes are given in table no. 2.

Surface study: Lower surface study of *M. charantia* leaf shows balloon shaped calcium carbonate deposition with various size. [Figure 1] The micro-measurement of the cystoliths are given in table no. 3.

***Lagenaria leucantha* Duch.**

Morphology: Leaves are shallowly 3-5 lobed, broadly ovate, pubescent, petiolate, sub-peltate. Lamina base deeply notched. Petiole long with striations, pubescent and 5.4 cm to 9.8 cm in length, Upper surface parrot green, glabrous and lower surface light green and measures about 4.2-13.8 × 4.6-13.1 cm. [Figure 2]

TS of leaf: Transverse section of *L. leucantha* leaf passing through midrib shows multicellular warty covering trichome, sessile glandular trichome and Multicellular trichome. Unicellular stalk with multicellular head was present in TS of midrib as well as petiole. [Figure 2] The micro-measurement of the trichomes are given in table no. 2.

Surface study: Lower surface study of *L. leucantha* leaf shows irregular shaped calcium carbonate deposition with various size. [Figure 2] The micro-measurement of the cystoliths are given in table no. 3.

***Luffa acutangula* L**

Morphology: Leaves are broadly ovate, acuminate, entire or 3-7 lobed, membranous, glabrous, dark green in colour and measures about 2.4-13.4 × 2.6-12.4 cm. Petiole long about 3 cm to 4.4 cm, ridged, with pulvinous base. [Figure 2]

TS of leaf: Transverse section of *L. acutangula* leaf passing through midrib shows multicellular stalk with multicellular head and glandular shaggy trichome while TS of petiole shows simple unicellular trichome and multicellular warty covering trichome. Multicellular stalk with unicellular head, sessile glandular trichome, multicellular warty trichome with shrunken cell and multicellular warty trichome were present in TS of midrib as well as petiole. [Figure 2] The micro-measurement of the trichomes are given in table no. 2.

Surface study: Lower surface study of *L. acutangula* leaf shows irregular shaped calcium carbonate deposition with various size. [Figure 2] The micro-measurement of the cystoliths are given in table no. 3.

***Cucumis prophetarum* L.**

Morphology: Leaves are broadly ovate, shallowly to deeply 3-5 lobed, scabridly hairy, upper surface light parrot green in colour and lower surface greenish yellow in colour with 3-5 prominent ribs, densely covered with trichomes and measures about 1.2-5.6 × 1.8-4.2 cm. Petiole long, striated, densely pubescent, measured about 1.5 cm to 3.5 cm. [Figure 2]

TS of leaf: Transverse section of *C. prophetarum* leaf passing through midrib shows multiseriate stalk with multicellular glandular head and multiseriate multicellular warty trichome while TS of petiole shows sessile glandular and pleuricellular trichome. Unicellular stalk with multicellular glandular head and multicellular warty covering trichomes were present in TS of midrib as well as petiole. [Figure 2] The micro-measurement of the trichomes are given in table no. 2.

Surface study: Lower surface study of *C. prophetarum* leaf shows irregular shaped calcium carbonate deposition with various size. [Figure 2] The micro-measurement of the cystoliths are given in table no. 3.

The different types of trichomes along with their measurements in particular samples are depicted in table no. 2.

From the six selected plant species of cucurbitaceae family almost all of them bears cystoliths in irregular shape except *M. charantia*, which possess balloon shaped cystoliths. Cystoliths, having smallest surface area presence in *L. cylindrica*, while the biggest one is from *C. prophetarum* up to 3.66µm^2.

The shape and surface area of cystoliths in different species of Cucurbitaceae family are mentioned in table no. 3.

Discussion

In Cucurbitaceae family, Calcified or silicified, Glandular capitates hairs with long or fairly long stalks types of trichomes are reported.[2] Unicellular trichome is exclusively found in *L. acutangula*. Warty multicellular trichome is found in all the plant samples under study expect *L. leucantha* where as multicellular trichome are found in *C. grandis*, *M. charantia* and *L. leucantha* but the length and width is variable in all the species which proves micrometry as important tool for distinguishing closely related plants. Pleuricellular trichome, multiseriate multicellular warty trichome and multiseriate stalk with multicellular head trichome which are exclusively found in *C. prophetarum*, which supports the reference.[2] Irregularly shaped cystoliths are found in all the plant samples under study expect *M. charantia* in which balloon shape cystoliths are observed. Variable size ranges of cystoliths helps in distinguishing the plants. In case of *C. prophetarum* surface area was found to be 3.66 µm^2 for irregularly cystoliths, which is biggest size for cystoliths as compared to all other plant samples.

On scientific observation, there were total 16 types of trichomes observed in present study from which 10 types of trichomes were non glandular while 6 types of trichomes

were glandular. The longest multicellular trichome and multicellular stalk with glandular head trichome is present in *M. charantia* measuring about 10.53-15.04 × 0.98-1.18 µm and 6.27-11.11 × 0.95-0.98 µm respectively. Multiseriate glandular trichome and pleuricellular trichome are present exclusively in *C. prophetarum* measuring about 1.66×0.65 µm and 2.3×0.51 µm respectively. Glandular shaggy trichome is present in *L. acutangula* only, which measures about 6.42×3.4 µm. Calcified trichomes is found in *L. cylindrical* measures about 4.92×2.77 µm and in *M. charantia* measures about 6.28×1.68 µm. Sessile glandular trichomes were absent in *C. grandis* and *L. cylindrica*. Multicellular trichomes with shrunken cell are present only in 3 species i.e. *L. cylindrica*, *M. charantia* and *L. acutangula*. The size of the longest trichome is 19.71-34.4×1.27-2.71 µm present in *M. charantia.*

Table 1: Histochemical tests

Reagent	Inference	Result
In dilute acid	Dissolved out, Cystolith remains a small, often stratified, basis composed of cellulose	++
In acetic, hydrochloric or sulphuric acid	Dissolves with effervescence	++

++ = positive result

Table 2: Presence of particular trichomes with measurements (40X×10X)

Types of trichome (µm)	*C. grandis*	*L. cylindrica*	*M. charantia*	*L. leucantha*	*L. acutangula*	*C. prophetarum*
Simple unicellular trichome	--	--	--	--	5.93×0.65	--
Multicellular trichome	6.74×1.71	--	10.53-15.04×0.98-1.18	5.32×4.42	--	--
Multicellular warty trichome	6.34-7.32×1.24- 1.56	4.44×2.7	6.91×1.35	--	4.35-8.87×0.85-1.96	4.6-8.01×1.33-2.25
Unicellular stalk with multicellular Head	--	--	--	2.13-2.69×0.59-0.83	--	1.86-2.45×0.54-0.66
Multicellular stalk with glandular head	3.6×0.76	2.77-2.92×0.62-0.75	6.27-11.11×0.95-0.98	--	2.26-9.02×0.69-2.13	--
Multicellular stalk with multicellular head	--	--	0.25-4.48×0.28-1.1	--	2.75-7.49×1.08-1.79	--
Multiseriate stalk with multicellular glandular head	--	--	--	--	--	1.66×0.65
Glandular shaggy trichome	--	--	--	--	6.42×3.4	--
Sessile glandular trichome (μm^2)	--	--	0.21	0.15-0.16	1.43-1.73	0.81
Multiseriate multicellular covering trichome	--	8.06×3.49	--	--	--	--
Multiseriate multicellular warty trichome	--	--	--	--	--	7.13-10.78×1.79-3
Calcified multicellular trichome	--	4.92×2.77	--	--	--	--
Warty multicellular covering trichome	--	3.67-6.21×1.38-2.21	--	2.83-8.14×1.42-5.95	3.65×1.7	1.37-8.9×0.33-1.87
Multicellular trichomes with shrunken cell	--	6.5×1.39	19.71-34.4×1.27-2.71	--	5.26-5.76×1.3-3.39	--
Pleuricellular trichome	--	--	--	--	--	2.3×0.51

-- = not found, µm=micrometer

Table 3: Shape and surface area of cystoliths

Plant	Shape	Surface area
C. grandis	Irregular	0.36-1.54 μm^2.
L. cylindrica	Irregular	0.13-0.73 μm^2
M. charantia	Balloon	1.04-2.14 μm^2
L. leucantha	Irregular	0.23-2.35 μm^2
L. acutangula	Irregular	0.19-0.94 μm^2
C. prophetarum	Irregular	0.32-3.66 μm^2

µm=micrometer

4. CONCLUSION

Key characters like presence of unicellular trichome in *L. acutangula*., warty multicellular trichome is found in all the plant samples under study expect *L. leucantha* whereas multicellular trichome are observed in *C. grandis*, *M. charantia* and *L. leucantha* which are variable in length and width among all the species which proves micrometry as important tool for distinguishing closely related plants. Moreover the presence of balloon shaped cystolith is remarkable character of *M. charantia* helpful in discrimination from other members. This study can be helpful for standardization of members belonging to cucurbitaceae family and also helpful in identification of adulteration or substitution.

6. REFERENCES

1. Sharma O P. Plant Taxonomy, 2nd edition, Tata McGraw-Hill Education Private Limited, New Delhi, p. 330.
2. Metcalfe CR and L. Chalk. Anatomy of the Dicotyledons, oxford at the clarendon press, London, 1950, vol I, p. 684.
3. Evans W C. Deterioration of stored Drugs Trease and Evans Pharmacognosy, 16th edition, W.B. Saunders Ltd, London, 2009, p. 553,555.
4. Metcalfe C R and L. Chalk. Anatomy of the Dicotyledons, oxford at the clarendon press, London, 1950, vol II, p. 1015.
5. Pandey B P. Plant Anatomy, 5th edition, S. Chand & company Ltd., New Delhi, 1978, p. 145-147.

6. Evans W C. Deterioration of stored Drugs Trease and Evans Pharmacognosy, 16th edition, W.B. Saunders Ltd, London, 2009, p. 561.
7. Pandey B P. Plant Anatomy, 5th edition, S. Chand & company Ltd., New Delhi, 1978, p. 67,67.
8. Metcalfe C R and L. Chalk. Anatomy of the Dicotyledons, oxford at the clarendon press, London, 1950;2: 1346
9. Watt W M, C.K. Morrell, D.L. Smith, M.W. Steer. Cystolith development and structure in *Pilea cadierei* (Urticaceae). Annals of Botany 1987; 60(1): 71-84.
10. https://books.google.co.in/books?id=U47nCAAAQBAJ&pg=PA172&lpg=PA172&dq=introduction+of+cystolith&source=bl&ots=YxIYAYFVc1&sig=2zaFTidb2o9-vcuX5DMJKK6n5ZE&hl=en&sa=X&ved=0ahUKEwjVrpOz5YjYAhWHwI8KHU0PCVUQ6AEILjAB#v=onepage&q=introduction%20of%20cystolith&f=false 10th January 2018.
11. Switu J, C.R. Harisha, P. Bhupesh. Variations of trichomes in family acanthaceae w.s.r. to kalmegh – a micromorphological and micrometric study. International Journal of Universal Pharmacy and Bio Sciences 2013; 2(6):526-534.
12. Switu J, C.R. Harisha. Morphological, structural and micrometric study of cystolith of family acanthaeceae w.s.r. to kalmegh. International Journal of Green Pharmacy 2014;13-17.
13. Harisha C R, J. Switu. Pharmacognostical study on trichomes of solanaceae and its significance. Universal Journal of Pharmacy 2013; 02(01): 100-104.
14. Bendre A. Practical botany, 1st edition, Rastogi publication, Meerut, p. 1-12.
15. Singh G. Plant systematic. 2nd edition, Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, p. 64-72.
16. Wallis T E. Text book of Pharmacognosy, 5th edition, CBS Publishers & Distributors, New Delhi, 2002, p. 123-132, 210-215.
17. Khandelwal K R. Practical Pharmacognosy, 19th edition, Nirali Prakashan, Pune, 2008, p. 51.
18. Krishnamurty K V. Methods in the plant histochemistry, Vishwanandan Pvt. Limited, Madras, 1988, p. 1-74.

Conflict of Interest: None

Source of Funding: Nil