



CYSTOLYTH AN INCREDIBLE JEWELS OF MEDICINAL PLANTS OF SOME FAMILIES-A SCIENTIFIC STUDY

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ABSTRACT

The type, morphology, and distribution of Calcium oxalate and Calcium carbonate crystals in mature leaves of five species of five families were studied. All the studied species contain calcium carbonate crystals. Species with both calcium oxalate and calcium carbonate. The calcium oxalate crystals were mainly found as druses or prismatic crystals. Druses were located in the crystal cells of both mesophyll and bundle sheath, but prismatic crystals were found only in cells of the bundle sheath. All calcium carbonate cystolyths were located in the epidermal lithocysts, and the types of lithocysts were related to the number of epidermal layers.

Keywords: Calcium carbonate crystals, Cystolyth, Leaves, Pharmacognosy.

INTRODUCTION

The Calcium oxalate crystals are formed frequently in lower plants and aquatic plants. They are deposited generally on the plant outer surface or in the intercellular spaces. In many plant species calcium crystals are commonly formed under ordinary conditions. These crystals are structural components in the leaves of many higher plant families. Their type and location are often used in plant taxonomic classification. Calcium oxalate is the most prominently deposited calcium salt. The crystals may occur in different plant organs and in various shapes, e.g. druses, prismatic crystals, raphides, styloides, and crystalands. However, Calcium carbonate crystals are found only in a few families such as Moraceae, Urticaceae, Cucurbitaceae, Cannabinaceae, Acanthaceae and in some of the Combrataceae and Boraginaceae. Well formed cystolyths are seen in the enlarged upper epidermal cells, dissolves in acid⁴. The cystolyths are structures combining wall material, including cellulose and callose, with calcium carbonate⁵. In a preliminary investigation of the Moraceae, we found both calcium oxalate and carbonate crystals, which encouraged us to study the specific distribution of differently shaped calcium carbonate crystals in mature leaves of selected species from five different families.

MATERIALS AND METHODS

Collection:

Collection of five samples of five different families leaves were been made as per collection standards⁶.

Morphology:

Leaves characters such as shape, size, base, margin, venation etc. are scientifically studied as per taxonomy⁷.

Pharmacognostical evaluation:

Transverse sections:

Free hand transverse sections of five leaves through midrib were taken. First studied with distilled water then studied stained with phloroglucinol and conc. HCl, microphotographs are taken by using corlzeisstrinocular microscope attached with camera⁸. The acid-etching test was used to identify the chemical compositions of crystals⁹.

Observation of Cystolyth:

For the observation of cystolyths, leaves studied through transverse sections and also through surface study at distilled water based mountings.

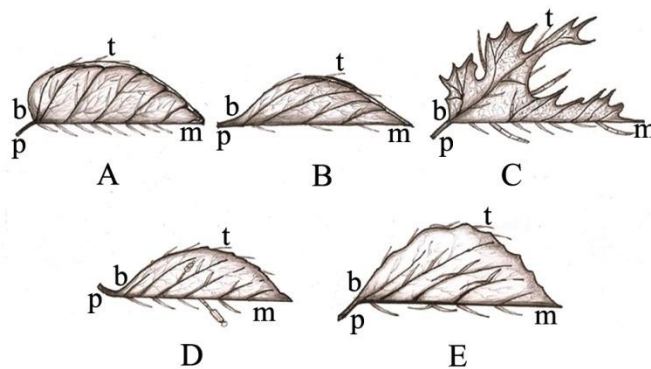
RESULT AND DISCUSION

Five species belonging to five families were selected for study (Table 1). They were collected during 2012. and identified. Morphological ray diagrams were scientifically represented in Plate No.1.

Sr. No.	Botanical Name	Sans. Name	Family
01	<i>Ficus benghalensis</i> L.	<i>Vata</i>	Moraceae
02	<i>Barleria prionities</i> L.	<i>Sahachara</i>	Acantaceae
03	<i>Momordica carentia</i> L.	<i>Karavelaka</i>	Cucurbitaceae
04	<i>Holoptelia intigrifolia</i> Pl.	<i>Chirabilwa</i>	Ulmaceae
05	<i>Cordia obliqua</i> W.	<i>Sleshmataka</i>	Boraginaceae

Table 1: Sample Description

Plate no. 1



t-trichomes, m-midrib, p-petiole, b- base.
 A- *F.bengalensis*, B- *B.prionitis*, C- *M.charantia*,
 D- *H.integrifolia*, E-*C.obliqua*.

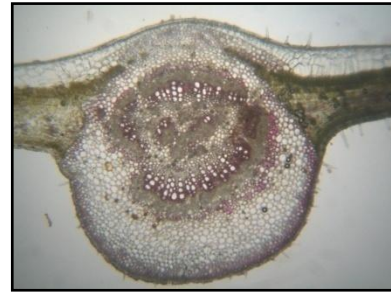
Plate No 2:Micro photographs



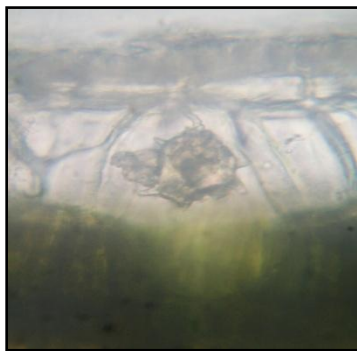
A. *Ficus bengalensis*



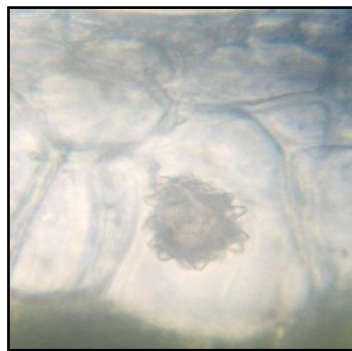
A.1-T.S Through midrib



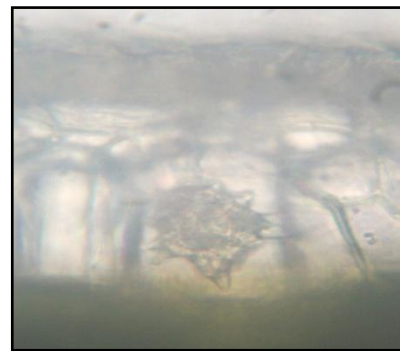
A.2-T.S Through midrib stained



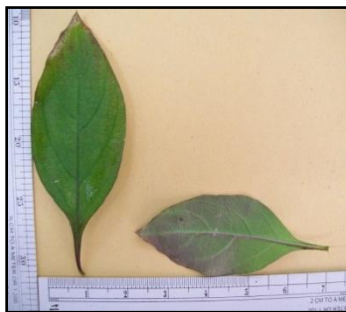
A.3-Cystolyth-upper epidermis



A.4- Cystolyth- sharp edges



A.5- Cystolyth- stalk



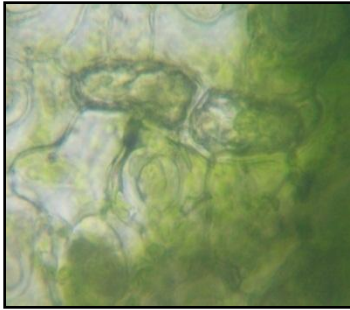
B.-*Barleria prionitis*



B.1-T.S Through midrib



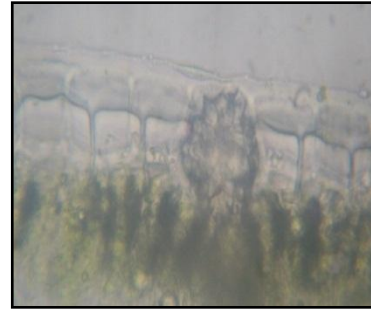
B.2-T.S Through midrib stained



B.3 Paired cystolyth



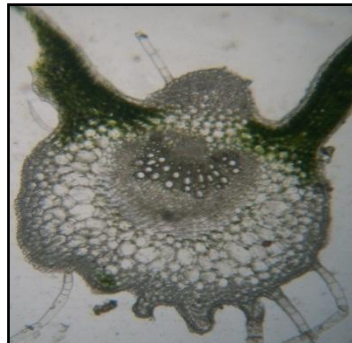
B.4-Cystolyth- lower epidermis



B.5- single cystolyth



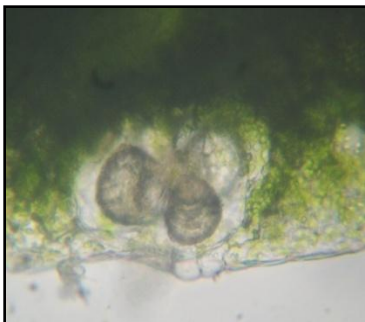
C- *Momordica carentia*



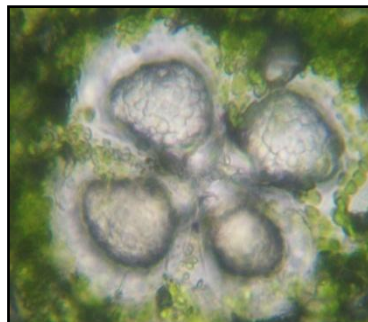
C.1-T.S Through midrib



C.2-T.S Through midrib stained



C.3- Tetra Cystolyth



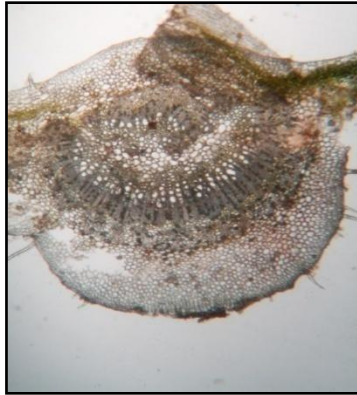
C.4- Cystolyth without sharp edges



C.5- cystolyth at lower epidermis



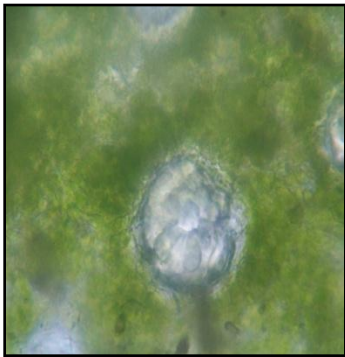
D. *Holoptelia integrifolia*



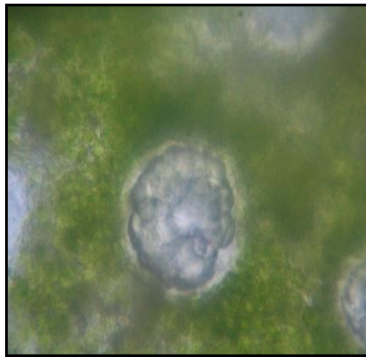
D.1-T.S Through midrib



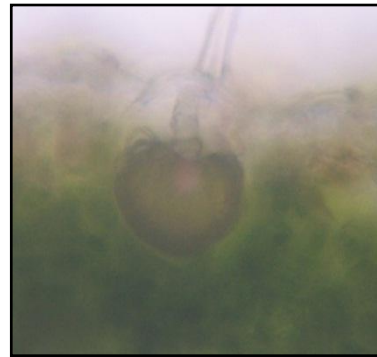
D.2-T.S Through midrib stained



D.3- Single circular cystolyth



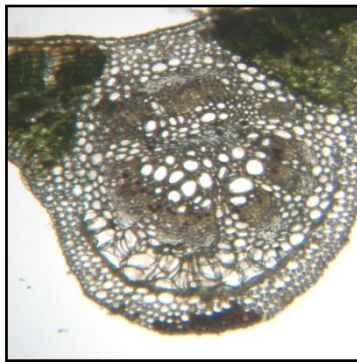
D.4- Cystolyth blunt surface



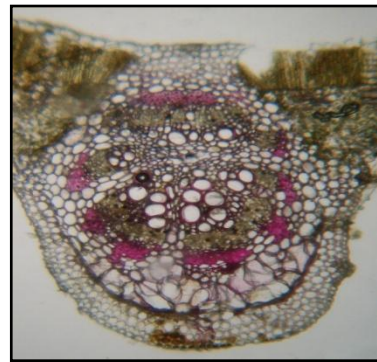
D.5- Cystolyth with stalk



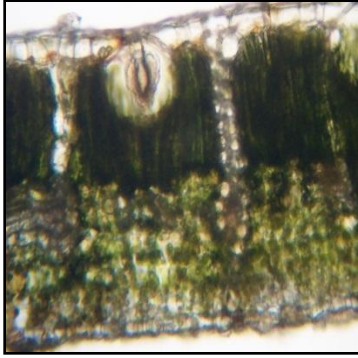
E-*Cordia obliqua*



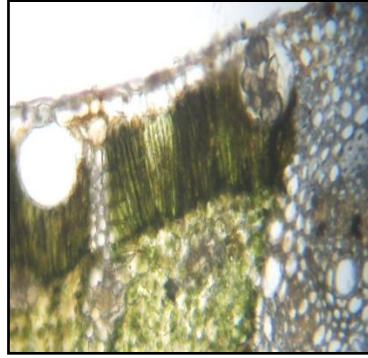
E.1-T.S Through midrib



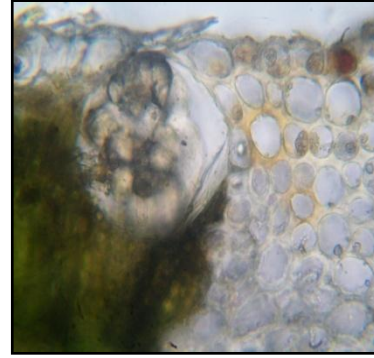
E.2-T.S Through midrib stained



E.3 Cystolyth upper epidermis



E.4- Dissolved cystolyth



E.5- Cystolyth blunt surface

Ficus benghalensis, L:

Leaves simple alternate, petiolate, petiole measures about 6x14cm, stipulate, stipule early withering, measuring about 4x8 cm, young leaves covered within stipules (vatashringi), stipules fleshy coloured in initially later on turns in to pale yellow, leaf ovate, margin simple, lamina measures about 6x12cm, lamina base cordate to subcordate, dark green above light green below, smooth epidermal hairs present over lower surface, midrib strong at lower surface lateral veins 4-5 and veinlets tended to meet margin of the leaf, many simple trichomes were scattered on both surface. Plate No. 1, A, Plate. 2. Fig. A.

T.S. of leaf:

Transverse section through midrib shows upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and some simple trichomes on both surfaces. Lamina shows upper 2-3 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma. Through midrib shows vascular bundle circularly arranged, bicollateral, some of meristele (2-3) located in the pith region. Vascular bundle surrounded by pericyclic fibres, rest of consists parenchyma cells. Vascular bundle surrounded by thick walled 3-4 layers of sclerenchyma cells. Plate. 2. Fig. A1-A2.

Cystolyth:

Cystolyths were initially originated in upper surface of the leaf and become elongated between the epidermal cells and sometimes between the epidermis and the palisade tissue throughout the section. Above lithocysts neither stoma nor trichomes was observed. Lithocysts are appearing like bunch of grapes inside the cells with prominent stalk. Cystolyth measures about 180 x 65 μm . The bunch hanged by the stalk crystals were over lapped and with sharp edges. When treated with Conc. HCl. immediately dissolves with effervescence forming empty space. Plate. 2. Fig. A3-A5.

Barleriaprionities, L:

Leaves simple opposite, sessile, measures about 4x9.5cm stipulate, leaf ovate, margin simple, lamina measures about 4x8cm, dark green above light green below, smooth epidermal hairs present over lower surface, midrib strong at lower surface lateral veins 4-5 and veinlets tended to meet margin of the leaf, many simple trichomes were scattered on both surface. Plate No. 1. B, Plate. 2. Fig.B.

T.S. of leaf:

Transverse section through midrib shows upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and some simple trichomes on both surfaces. Lamina upper 2 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma loaded by chloroplasts. Through midrib shows bicollateral vascular bundle. Vascular bundle surrounded by thick walled 2-3 layers of sclerenchyma cells. Plate. 2. Fig.B1-B2.

Cystolyth:

Cystolyths were initially originated in upper surface of the leaf between the epidermal cells and sometimes between the epidermis and the palisade tissue throughout the section. Initially cystolyths form at the upper epidermis form rounded structure and later on give two oppositely elongated balloons like structure with prominent stalk. Cystolyth measures about 160 x 60 μm . The bunch hanged by the stalk crystals were overlapped and without sharp edges. When treated with Conc. Hcl. immediately dissolves with effervescence forming empty space. Plate. 2. Fig.B3-B5.

Holopteliaintigrifolia, Pl:

Leaves simple alternate, petiolate, petiole measures about 6x10cm stipulate, stipule early withering, measuring about 0.3-0.5 cm. petiole twisted and forming light channel on upper surface, stipules two on both surface of petiole, leaf ovate, in young leaves margin serrate later on leaf matures base become simple while end somewhat crenated serrate margin, lamina measures about 6x8cm, dark green above light green below, smooth epidermal hairs (simple and glandular present) over lower surface, midrib strong at lower surface lateral veins 4-5 and veinlets strongly network finally divided and reach margin of the leaf, many simple trichomes and glandular present were scattered on both surface. Plate No.1. D, Plate. 2. Fig.C.

T.S. of leaf:

Transverse section through midrib shows upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and some simple and multicellular glandular trichomes on both surfaces. Lamina upper 2-3 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma. Through

midrib shows vascular bundle discontinuous circular ring arranged, bicollateral, centrally located in the pith region. Vascular bundle surrounded by pericyclic fibres, rest of consists parenchyma cells. Vascular bundle surrounded by thick walled 2-3 layers of sclerenchyma cells. Plate. 2. Fig.C1-C2.

Cystolyth:

Cystolyths were initially originated in lower surface of the leaf throughout the section. Initially cystolyths form at the lower epidermis form rounded structure and later on become mushroom like structure with prominent stalk. Cystolyth measures about $140 \times 35 \mu\text{m}$. The bunch hanged by the stalk crystals were overlapped and without sharp edges. When treated with Conc. Hcl. immediately dissolves with effervescence forming empty space. Plate. 2. Fig.C3-C5.

Momardiccharantia, L:

Leaves simple alternate, petiolate, petiole measures about 2-4 cm exstipulate, leaf ovate, marginedeely lobed lobes 3-4, lamina measures about $8 \times 10 \text{cm}$, lamina base cordate to subcordate, dark green above light green below, smooth epidermal hairs present over lower surface, midrib strong at lower surface lateral veins 4-5 and veinlets tended to meet margine of the leaf, many simple trichomes were scattered on both surface. Plate No.1. C, Plate. 2. Fig.D.

T.S. of leaf:

Transverse section through midrib irregular in shape and shows upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and some simple and multicellular pointed trichomes on both surfaces. Lamina upper one or two layered palisade parenchyma and lowers 2-3 layers of spongy parenchyma. Through midrib shows vascular bundle radially arranged, upper xylem and lower phloem Vascular bundle surrounded by thick walled 2-3 layers of sclerenchyma cells. Plate. 2. Fig.D1-D2.

Cystolyth:

Cystolyths were initially originated in lower surface of the leaf throughout the section. Initially cystolyths form at the lower epidermis form rounded structure and later on divided into two, three and also upto five balloons like structure without prominent stalk. Each cystolyth measures about $270 \times 50 \mu\text{m}$. The bunch hanged by the stalk crystals were overlapped and without sharp edges with airfilled lungs like structure. When treated with Conc. Hcl. immediately dissolves with effervescence forming empty space. Plate. 2. Fig.D3-D5.

Cordia oblique, W:

Leaves simple alternate, petiolate, petiole measures about 6x12cm exstipulate, leaf ovate, margine base simple at the tip serrate to dentate, lamina measures about 6x11cm, dark green above light green below, rough leathery, smooth epidermal hairs present over upper and lower surface, midrib strong at lower surface lateral veins 4-5 and veinlets tended to meet margine of the leaf, many simple and bilobed sessile trichomes were scattered on both surface. Plate No.1. D, Plate. 2. Fig.E.

T.S. of leaf:

Transverse section through midrib shows upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and some simple and bilobed trichomes on both surfaces. Lamina upper 2-3 layered palisade parenchyma and lower 5-6 layers of spongy parenchyma. Through midrib shows vascular bundle circularly arranged centrally forming pith. Vascular bundle surrounded by pericyclic fibres. Xylem present above the phloem, rest consists parenchyma cells. Vascular bundle surrounded by thick walled 3-5 layers of sclerenchyma cells. Plate. 2. Fig.E1-E2.

Cystolith:

Cystoliths were initially originated in upper surface of the leaf between the epidermal cells and sometimes between the epidermis and the palisade tissue throughout the section. Initially cystoliths form at the upper epidermis form rounded structure and later on give linear elongated balloons like structure with prominent stalk. Cystolith measures about 150x40 μm . The bunch hanged by the stalk crystals were overlapped and without sharp edges. When treated with Conc. Hcl. immediately dissolves with effervescence forming empty space. Plate. 2. Fig.E3-E5.

DISCUSSION

Calcium crystals were observed in all plants investigated (Tables 1). The morphology of cystolith and the distribution of lithocyst are genera and species specific in family acanthaceae¹⁰. Crystals in moraceae are commonly described in taxonomic literature. Cystoliths were never occupy the whole cell, slightly detached and hanged over by stalk. There are 8 genera and 49 species of moraceae in Taiwan¹¹. However, there is a shortage of information in the literature on this particular relationship. The presence of crystals is certainly not detrimental to the plant. Physical and chemical conditions, such as temperature, pressure, pH, and ion concentration, may affect crystal growth, habit, and properties¹², but the precise controlling mechanism for crystal formation in plants is still unknown. Factors which control oxalate synthesis and cellular calcium uptake and mobility may affect crystal induction and formation¹³. The presence or absence of crystals is one of the important characters for understanding the evolutionary relationships of the plant species¹⁴.

CONCLUSION

Article has highlighted numerous results about cell-mediated crystallization of calcium carbonate in plants. Cystoliths are very important role in identification of discussed plants, in systematic, scientific identification character in which identified the microscopical family characters to overcome from the species by studying the shape and size of the cystoliths. Future research in this area will benefit from applying a variety of integrated approaches. There is a critical need for correlative biochemical and biophysical characterization, which may entail traditional approaches such as organelle and membrane isolation and characterization.

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