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MICROMORPHOLOGY OF CALCIUM OXALATE CRYSTALS IN SELECTED MEDICINAL PLANTS

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ABSTRACT

Calcium oxalate crystals, one of the major ergastic constituents excreted in the plant cells, have been subjected to intensive studies by many pioneer investigators. The crystal profile of the plant organ was said to be inconsistent in the sense that no specific crystal habit can be assigned to a particular species. However, a comprehensive survey of crystal habits in several medicinal plants, where crystal deposition is very high, was carried out. The result of the present study lends support to the application of crystals in the Botanical diagnosis of crude herbal drugs. Polarized light microscopy considerably enhances the utility of crystal morphology and topography in herbal drug standardization.

KEYWORDS: Calcium Oxalate crystals, ergastic constituents, polarized light, herbal drug, standardization.

INTRODUCTION

The Calcium oxalate crystals in plants were observed in various crystal habits; their generalized shapes and aggregates have been described (Metcalfe & Chalk 1950; Prychid & Rudall 1999; Franceschi & Horner 1980). The exomorphic features of calcium oxalate crystals usually is consistent and specific within a given plant species and often within a given taxonomic group (Arnott & Pautard 1970) crystal size can also tremendously. This is partly a function of the cell type in which it formed the amount of available calcium and other environmental factors. Characters such as crystal hydration state (monohydrate & dihydrate), the ratio of calcium to oxalate, the presence of nucleating substances or contaminants and involvement of specialized cellular structure, all play a role in determining crystal morphology (Franceschi & Nakata 2005).

MATERIALS AND METHODS

Medicinal plants were selected from several families, collected during flowering season, and immediately preserved in FAA (Formalin – Acetic acid – Alcohol: 5 ml +5ml + 90ml of 70% Alcohol) for 24 hrs and processed by standard paraffin procedure (Berlyn and Mikshe, 1976) sections were cut 10 µm thick and stained (Price 1967) for polarized light microscopy. Some of the specimens were dehydrated employing Tertiary Butyl Alcohol (TBA) series as per the schedule published Sass (1940); following dehydration, the specimen were subjected to paraffin wax infiltration and cast into wax blocks. Serial sections of 10-12 µm thickness were prepared by Rotary microtome. Histochemical determination of crystals as calcium oxalate was done on similar sections, using the procedure of Yasue (1969) and viewed with bright field light microscope.

The Plant organs were processed and bleached (5% Sodium hypochlorite) and washed with water and ethanol dehydration series, then to xylol and mounted and unstained. They were examined under full and partial polarizers and captured digitally. Free hand sections made by razor blade were also observed and captured digitally.

RESULTS

Five types of calcium oxalate crystals were identified. They are prismatic, sphaerocrystals, rosettes, raphides and micro crystals.

These five type of crystals were observed in the stem, lamina, petiole, leaves, scaly leaves, bark, wood and roots of *Aegle marmelos, Aerva lanata, Allium cepa, Asparagus racemosus, Begonia malabarica, Boerhavia diffusa, Bridelia crenulata, Carissa spinarum, Datura innoxia, Fluggea virosa, Indigofera tinctoria, Jatropha gossypifolia, Justicia adhatoda, Limonia acidissima, Morinda tinctoria, Phyllanthus polyphyllus, Terminalia chebula, Thespesia populnea, Tribulus terrestris.*

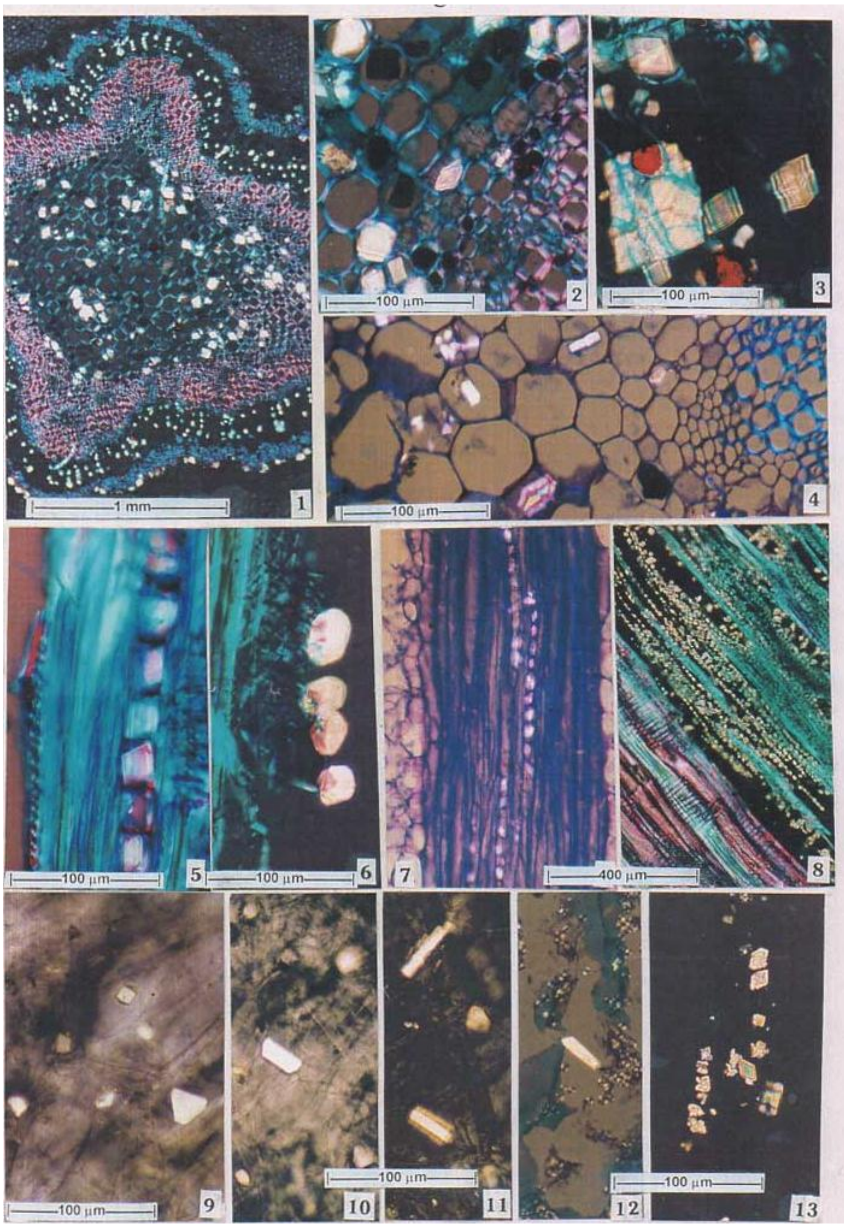

Fig. 1.1 - 1.13

Prismatic Crystals

Simplest form of calcium oxalate crystals are **prismatic type** which may either be solitary or in twinned state. They vary in shape from rectangular to pyramidal; they come under either **tetragonal** (three axes at right angles to one another) or monoclinic (three unequal axes with two lateral axes at right angles to one another, but only one of these at right angles to the third axis).

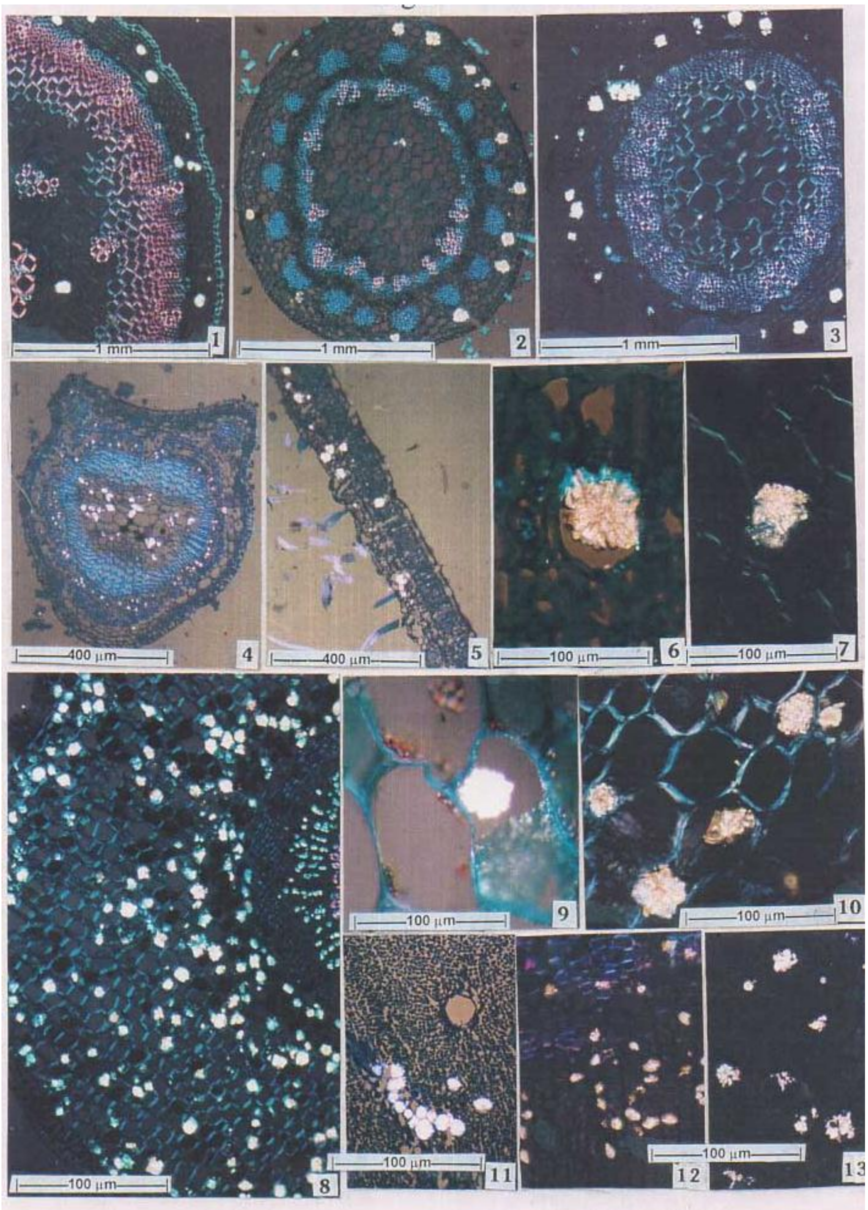

Fig. 2.1 - 2.13

The prismatic crystals usually occur in a mosaic pattern of different shape and size in the cortex, pith and parenchyma cells of the wood and bark. Scaly leaves of *Allium cepa* bulb contains dense mat of almost all habits of prismatic crystals (Fig.1.9, 10, 11). In *Bridelia crenulata*, the ground tissues of pith and cortex bear abundant prismatic crystals (Fig.1.1, 2, 3) In leaves, wood and bark, it was observed that the prismatic crystals were invariably associated with sclerotic axial elements, apart from other types of crystals found in the ground parenchyma, Eg. *Aegle marmelos* (Fig. 1.7); *Justicia adhatoda*, (Fig.1.4 & 5), *Limonia acidissima*, (Fig. 1.8); *Terminalia chebula* fruit (Fig. 1.12); and *Phyllanthus polyphyllus* (Fig. 1.13).

Sphaerocrystals (DRUSES)

More prevalent category of crystals widespread in plants is the **Sphaerocrystals** or **druses.** These crystals occur usually in the ground parenchyma without any specific affinity towards particular location in the plant organs. Sphaerocrystals is agglomeration of numerous individual prisms or pyramids clinging together to assume the shape of the spiny ball (Fig. 2.6, 7, 8, 9). Exclusive occurrence of druses was recorded in *Aerva lanata*, *Fluggea virosa* (Fig. 2.10), *Tribulus terrestris* (Fig. 2.2), *Indigofera tinctoria* (FIg. 2.4), *Jatropha gossypifolia* (Fig. 2.13) etc. Eventhough the druses are nonspecific in their locations in the plant cells, very often they are exclusive in the phloem rays of many plants, eg; *Thespesia.*

Rosette crystals apparently simulate the sphaero crystals, but differ in that they have a very wide central organic part form which several pointed crystals radiate equidistantly all around (Fig. 3) Rosette crystals occur in stem of *Justicia adhatoda,* (Fig. 3.10) and *Carissa spinarum* (Fig. 3.9).

Acicular crystals: These are slender long needles with pointed ends. The needles are usually aggregated into a bundle called **Raphides**. Exclusive crystal type of raphides occurs in the phloem rays of *Morinda tinctoria,* (Fig. 3.4 & 5); cortical parenchyma of *Asparagus racemosus,* (Fig. 3.8); *Boerhavia diffusa* (Fig. 3.6 & 7) etc.

Microcrystals or **sand crystals**: These are very minute crystals occurring in large numbers filling the entire cell lumen. The form of individual crystals may range from small prisms, monoclinic microspheroides and tetragonal microspheroids. Sand crystals were observed in *Datura innoxia* (Fig. 3.3) and *Begonia malabarica* (Fig. 3.1 & 2).

Fig. 3.1 - 3.10

DISCUSSION

Chattaway (1955) in her pioneer studies on crystals in wood tissues had highlighted the validity of application of crystal morphology in wood identification. This contention was based on the finding that the crystals were distinct in appearance and relatively infrequent in occurrence. However, Scurfield (1973) *et al.* were sceptical of this tenet and believed that to imply taxonomic values, the crystal habits must have some genetical basis. They further expressed that the crystals assumed multitude of habits and more than one morphological category of crystals occurred in the adjacent cells. It is partly true that the crystal profile of an organ may assume a mosaic pattern of several morphological categories. However, there are many instances where only one type of crystal predominates in the species. To cite examples where the phenomenon of specificity of crystals is expressed in toto, in the secondary phloem of *Morinda tinctoria*, (Fig.3.4 & 5) cortical tissue of *Asparagus racemosus* (Fig. 3.8) and in the mesophyll tissue of *Boerhavia diffusa*, (Fig 3.6 & 7); the raphides are the only crystal type. So also the crystal sand seems to be restricted in a few dicotyledons, eg; *Solanaceae* (Wallis, 1985). The axial parenchyma components of secondary phloem of many plants are exclusively loaded with druses. It was also found that prismatic crystals of various habits were invariability associated with sclerenchyma elements in the leaves, secondary xylem and secondary phloem. Such localized distribution of prismatic crystals evokes interests in the biochemical correlations of lignification and crystal deposition. Occurrence of styloid type of crystals in the *Rutaceae* and star crystals in the seed coat of *Umbelliferae* are few more instances of crystal specify *vis – a – vis* taxonomic values.

CONCLUSION

Another interesting phenomenon that came into light during the present investigation is that most of the plants which are claimed to possess therapeutic values are invariably associated with a high deposition of calcium oxalate crystals. This observation lends to presume that calcium oxalate in plants have some definite role to play in the clinical activities.

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