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Research Article – Plant Science

Crystallomorphic diagnosis of medicinal plants and their adulterants

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Abstract

The global level revival of enthusiasm in the alternative system of therapy, especially the renewed trends towards the herbal based indigenous formulations, has landed in the execution of certain mandatory measures. Of several such precautionaries, detection and prevention of intentional adulteration, maintenance of quality control of the herbal drugs assume primary exigence. Botanical determination of the genuineness of the herbal drugs, beyond any doubt, is the primary step before subjecting the drugs for pharmaceutical evaluations. Several botanical techniques are accessible to authenticate the identity of the herbal drugs, and application of crystallomorphic divergence is a simple but effective strategy for checking the original drugs from their adulterants. Calcium Oxalate Crystals are accredited with the phenomeon of pleomorphism and the physical property of birefringence. Added to it, the crystal habits are restricted in distribution among the plants and sometimes, specific to certain Angiosperm taxa. Further, the crystals in plant cells are easy to detect microscopically, especially under polarized light. On these merits, the morphological dimensions of the crystals and their dynamics in relation to different plant groups offer a helping hand in herbal drugs standardization. The present paper highlights the crystallomorphic spectrum and gamut of variation of the crystal scenario among the medicinal plants. The major types of crystal include prismatic, spiny spheroids or the druses, smooth spheroides (polyhedrals), rosettes, styloids, solitary acicular, bundled acicular or raphides and sand or microcrystals. A new crystal type, namely, scalloped crystal has been recognised and added to the list. Citing specific crystal habits form the survey, a protocolic technique of application of crystallography in identification of important medicinal plants and detecting their adulterants is presented. The elite of those medicinal plants drawn from the list of renowned herbals used in ISMO are opted to illustrate the crystal specificity in relation to drug identity.

Key words: Calcium oxalate crystals; birefringence; polarised light; herbal drug standardization

Introduction

In plants, cellular activities not only release energy for growth and differentiation, but also build up reserve and waste materials which are collectively called ergastic substances. Among these reserve cell contents, the crystals of diversifed morphological categories and chemical composition are most prominently represented in plant tissues. Calcium carbonate occurs in a few selected families like *Moraceae, Urticaceae, Cucurbitaceae* and *Acanthaceae*, Calcium oxalate dominates any other crystal types both in frequency and morphological habits. Calcium oxalate crystals have been estimated to account for 1-20% of dry weight of a plant (Evans, 1996). The size of the crystals and the morphology they adopt are belived to the related to the size and shape of the cells in which they are formed (Scurfield *et al.*, 1973). The crystals may occur in simple cells not different from the neighbouring cells or in idioblasts which are usually dilated and larger than the neighbouring cells.

The chemical nature of the calcium oxalate can be tested and used for diagnostic purposes. They are insoluble in water, alcohol and acetic

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acid; they will dissolve, however, in hydrochloric acid, sulphuric acid and nitric acid.

Albeit spectrum of morphological habits and the gamut of topographic variations, the crystallographic attributes of medicinal plants have not been given due accent in the pharmacopoeial standard. In addition to taxonomic applications, as stressed by Chalk (1983), Chattaway (1955) and many other pharmacogocists, morphology, localisation, density and other features of the crystals may be effectively exploited for establishing the identity of crude drugs of plant origin. The pressent paper higlights these lines of thinking of the crystals in plants. Literature on various dimensions of crystallography is really voluminous. Soleredor (1908) in his masterly treatise of plant anatomy has given an accurate description of crystal habits and their occurrence in plants. Chattaway (1955, 1956) made a comprehensive survey of crystals of about 1000 genera and 160 families. His work is more related to wood tissues rather than other organs of the plant. Metcalfe (1983) has given more detailed account of plant crystals and dealt with their classification, distribution, origin and taxonomic applications. Franceschi *et al.* (1980) reviewed in an elaborate way the synthesis of oxalic acid and building mechanism of calcium oxalate crystals. They also presented all available literature concerned with physiology of calcium Oxalate crystals in plants and animals and their SEM pictures (Vijayasankar Raman et al., 2014) of different forms of crystals in plants are exemplary. Most of these papers highlight the value of crystals in the taxonomic treatments of plants (Christiana J. prychid.1999). Pharmacognocists (Wallis, 1985: Evans, 1996) pay much importance for the crystals especially while dealing with identify of powder drugs of plants. Collection of morphological data of the crystals from plant tissues is simple and easier. So, crystallomorphic features could be fully exploited in the pharmacognostic studies. Many of the plant drugs employed in ISMO remain unexplored of their crystal profile. It is aimed in the present investigation to make a survey of some selected plants of medicinal importance with regard to their crystal contents and to evaluate the applicability of crystal morphology in the identification standards of the drugs.

Materials and Methods

Stained permanent rotary microtome Sections (Sass, J.E 1940) leaf tissues cleared with sodium hydroxide and powdered drugs were studied during the investigation. Observations were made both under ordinary light and polarised light. Polarization microscopy is based on the phenomenon of birefringence property of anisotropic substances. When substances like crystals are viewed under Polarised light microscope the object appears bright against dark background (Fig.1 *Aerva Lanata* stem and leaf, *Syzygium* leaf and Petiole). Shape, size and distribution of even minute crystals were detected under the device. Photomicrographs of the crystals were taken NIKON Lab phot 2 attached with analyser and polarizer plates. For description of crystals non-crystallographic morphological terms were used during observation. (Esau .K 1967)

Observation

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). 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 2.9, 10, 11). In *Bridelia crenulata*, the ground tissues of pith and cortex bear abundant prismatic crystals (Fig.2.1,2,3) In leaves, wood and bark, it was observed that the prismatic crystals were invariably associated with sclerotic axial elements, apart form other types of crystals found in the ground parenchyma, Eg.*Aegle marmelos* (Fig.2.7); *Adhatoda zeylanica* (Fig.2.4 & 5), *Feronia elephantum* (Fig 2.8); *Terminalia chebula* fruit (Fig2.12); and *Phyllanthus polyphyllus* (Fig.2.13).

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.

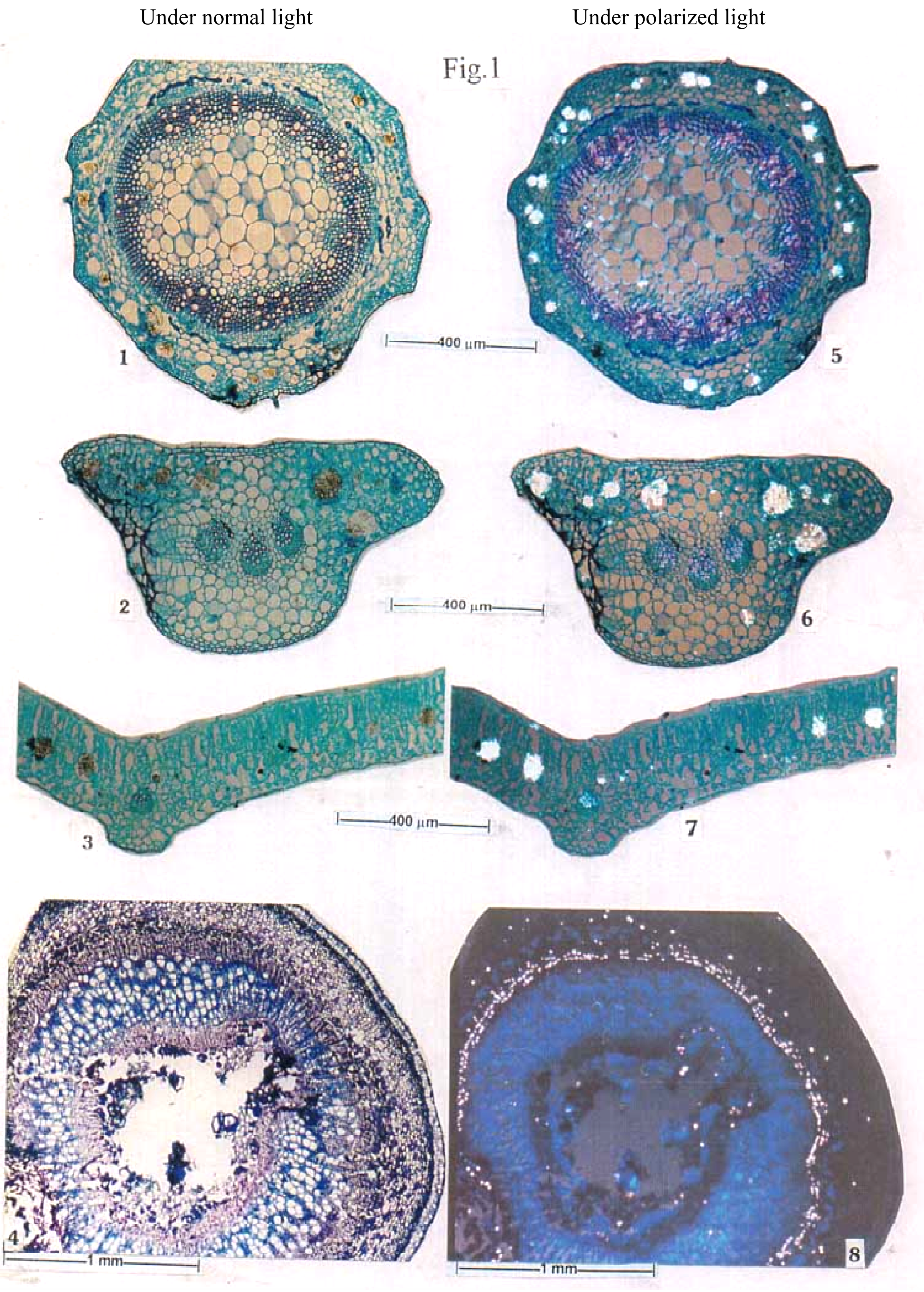
Under normal light
Under polarized light
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Fig.2

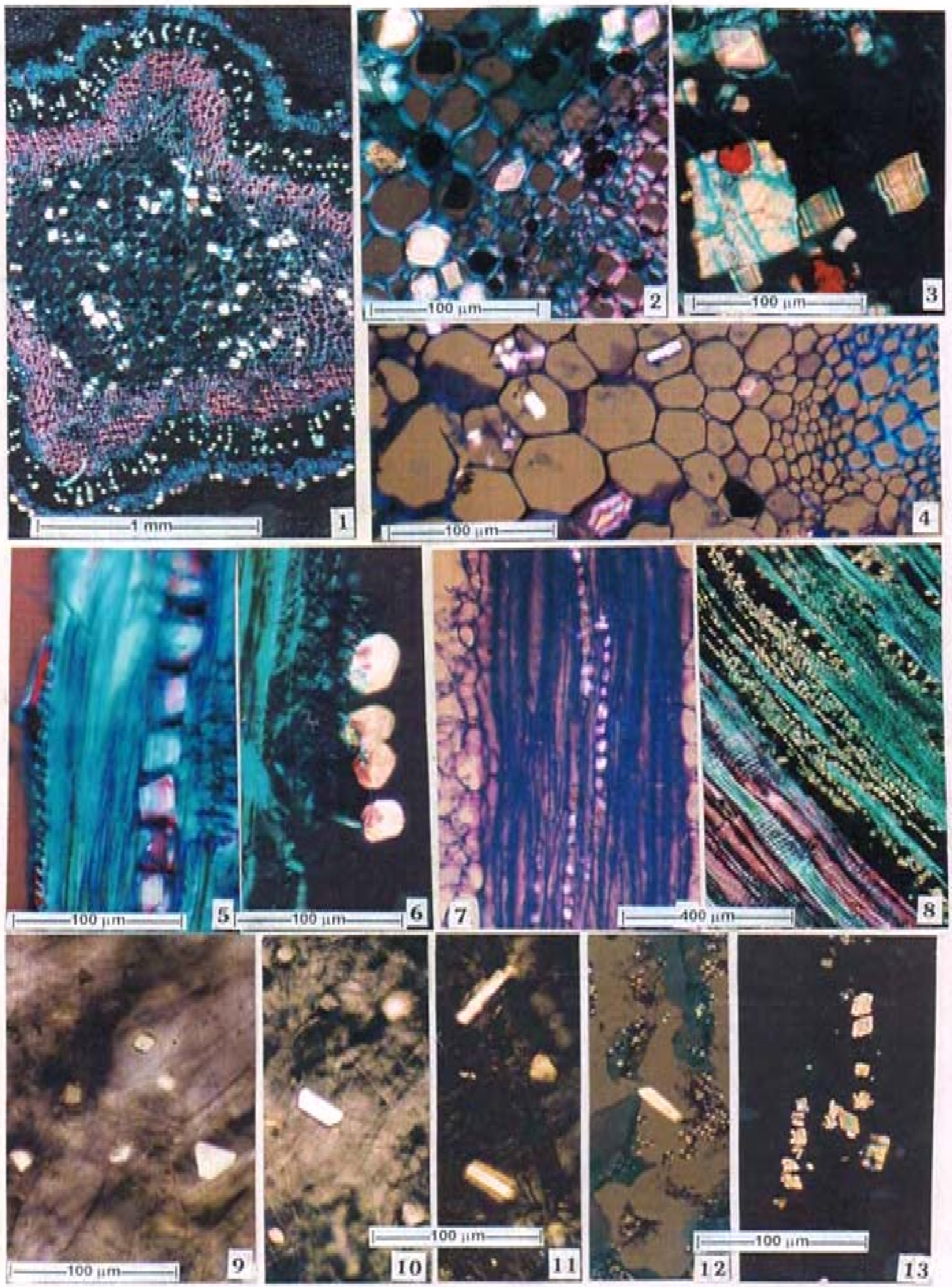
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Fig.3

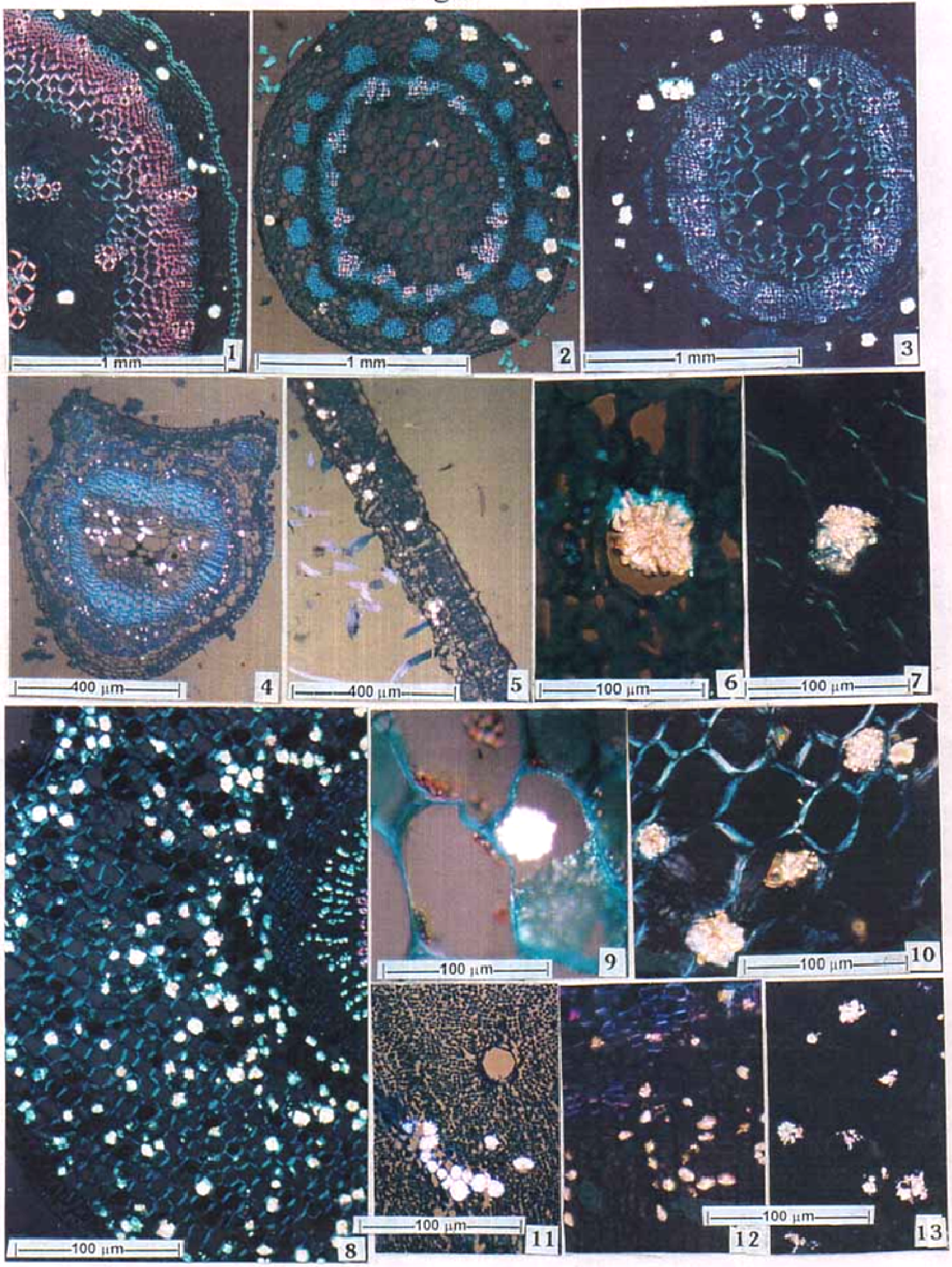
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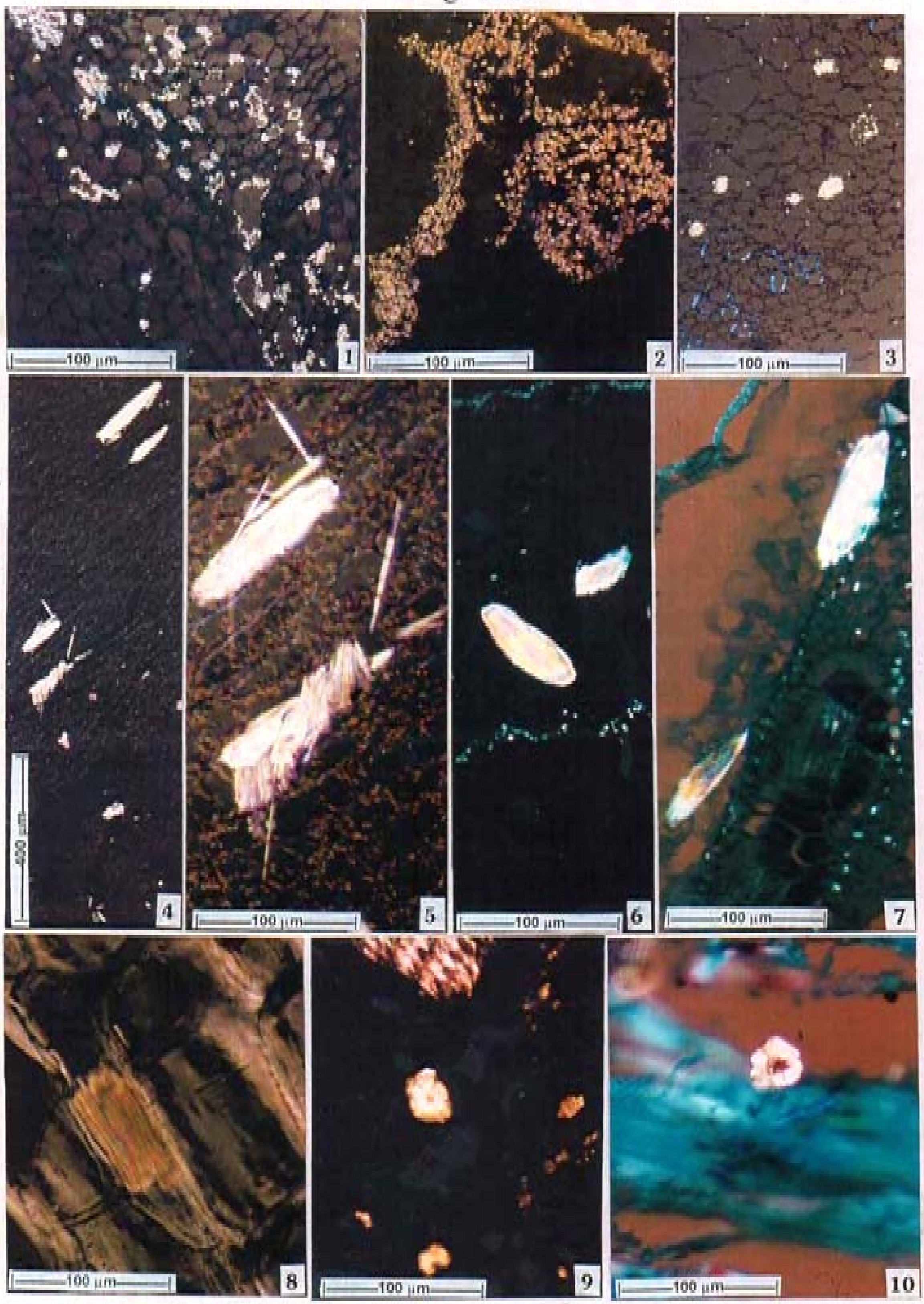
Fig.4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Sphaerocrystals is agglomeration of numerous individual prisms or pyramids clinging together to assume the shape of the spiny ball (Fig.3.6,7,8,9). Exclusive occurrence of druses was recorded in *Aerva lanata, Securinega virosa* (Fig.3.10), *Tribulus terrestris* (Fig.3.2), *Indigofera tinctoria* (FIg.3.4), *Jatropha gossypifolia* (Fig.3.13) 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;*Thepesia.*

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.4) Rosette crystals occur in stem of *Adhatoda zeylanica* (Fig.4.10) and *Carissa spinarum* (Fig.4.9)

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* (Fig 4.4,5); cortical parenchyma of *Asparagus* (Fig.4.8); *Boerhavia diffusa* (Fig.4.6,7) 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.4.3) and *Begonia malabarica* (Fig 4.1 and 2).

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 haved 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* (Fig.4.4,5) cortical tissue of *Asparagus* (Fig.4.8) and in the mesophyll tissue of *Boerhavia* (Fig 4.6 and 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 localised distribution of prismatic crystals evokes interests in the biochemical correlations of lignification and crystal deposition. Occurrerence of styloid type of crystals in the *Rutaceae* and star crystals in the seed coat of *Umbelliferae* are few more instances of crystal specifity *vis – a – vis* taxonomic values.

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 acitivities.

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