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## Ergastic Crystal Studies for Raw Drug Analysis

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### Abstract

Phytochemical constituents are distributed in various parts of plants and their localization is indicative of their therapeutic properties. Ergastic crystals such as calcium oxalate crystals are also found in almost all plant parts, which is an anti-nutrient as the dietary oxalates contributes to human ailments. Several of the medicinally useful plants contain these crystals and consumption of such plant materials in raw form can cause health problems in humans. Ergastic crystals can be an important diagnostic tool for the identification of raw drug as in *Costus pictus* a medicinal spiral ginger commonly called Insulin plant is devoid of cuboidal crystal but its related *Costus speciosus* leaves possess characteristic cuboidal shaped crystal in its leaf mesophyll. Gene manipulation technology may be promising in removing such deleterious genes or introduction of altered bio-chemicals to nullify such effects for the future generation.

**Keywords:** ergastic crystals, calcium oxalates, medicinal raw drugs

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### 1. Introduction

Plants are the storehouses of multifunctional components with nourishing, healing, refreshing, curing and replenishing qualities. Such chemical constituents are distributed in various parts of plants and their localization is indicative of their therapeutic properties. Even though plants can be classified into medicinal and non-medicinal there is no such distinction in traditional systems such as Ayurveda, which denote that the whole earth with its living and non-living entities have curative properties. Among the vast combination of phytochemicals that are useful for the consumer world there are certain deleterious chemicals or its combinations. The undesirable component in phytochemicals requires to be eliminated either by chemical treatments or simply avoiding its usage. Several of the plant resources are being used as

raw materials for medicinal drugs. Therefore the knowledge of ergastic crystals in food and medicinal raw materials and in finished products is expected to bring out furnished products for longevity.

## 2. Classification of ergastic crystals in plants

Classifications of cytoplasmic constituents at various levels are available. Ergastic substances represent waste products, which are solid and secondary. Of these secondary products are alkaloids, glycosides, tannins, volatile oil, resins, gums and mucilage. Solid products include calcium oxalate, calcium carbonate, hesperidin, diosmin and silica. Non-living inclusions are classified as ergastic substances [1]. Their categorization into secretory products, excretory products and reserve materials indicate their functional association. The secretory products include nectar, enzymes and coloring matter. Proteins, fats and oils, and carbohydrates represent reserve materials. Excretory products represent alkaloids, tannins, resins, latex, volatile oils and mineral crystals. The common mineral crystals are calcium oxalate, calcium carbonate and silica. According to Esau ergastic substances are products of metabolism the examples being, carbohydrates proteins, fats, tannins and various types of crystals [2]. They are mainly non-protoplasmic components distributed in the vacuoles, in the cell wall and associated with the protoplasmic components. Fahn considers ergastic substances as organic and inorganic by products of metabolism [3]. Crystals of inorganic compounds such as gypsum and silica are less common. Crystals of organic substances such as carotene, berberine and saponin are relatively common.

## 3. Shapes and size of ergastic crystals

Ergastic crystals are reported from almost all plant parts such as rhizome, corm, tuber, adventitious roots, leaves, fruits and even in seeds. Calcium oxalate exists in varying crystal shapes and sizes in plants, with raphides being the predominant crystal form [4–8]. Various types of calcium oxalate crystals exist in the form of prisms, acicular, raphides, clusters, rosettes etc. The shape of crystals may be cuboidal, rhomboidal, octahedral or elongated. Elongated crystals when massive and solitary are known as styloids as found in Iridaceae. When they are compound and cluttered in spherical masses they are called as druses. Small prismatic crystals as well as minute crystals are known as crystal sand. Special crystal containing cells are called idioblasts, which are cells that differ distinctly from surrounding cells in both shape and structure. Raphides are usually found in very large cells which when mature do not contain living protoplast, but are filled with mucilage. Raphides at maturity are dead structures usually filled with mucilage and are reported to be capable of swelling. Parts of the cell wall of such raphide idioblasts remain thin and if the mucilage swells, the thin wall bursts and the raphides are ejected.

Idioblasts with raphides are found in many monocots and also in some dicots such as in the petals of *Impatiens balsamina*. Silicon salts are often deposited in cell walls as is common in the grasses but they can also be found within the cell. Cystoliths are internal outgrowths of cell

wall that are encrusted with Calcium Carbonate or impregnated with minerals. They occur in ground parenchyma and epidermis. In epidermis, they may be formed in hairs, or in special enlarged cells, the lithocytes.

Silica is deposited mostly in cell walls, but sometimes it forms bodies in the lumen of the cells. Poaceae the grass family is a well-known example of the plant group having silica in both the walls and cell lumen [3].

#### **4. Histochemical methods for observation of ergastic crystals**

For the temporary mount preparation of free hand sections fresh or preserved materials can be used for light microscopic study that reveals large sized crystals. Russell classifies the light microscopic study for the calcium determination in two groups [9]. They are metal substitution technology and dye lake reactions. Calcium oxalate identification is done by various methods including light microscope, polarizing optics and scanning electron microscopic (SEM) studies. Yasue histochemical method is highly efficient as it can localize calcium oxalate even in plant trichomes [10, 11]. SEM studies reveals crystals of very small size. The application of X-ray diffraction technology and infrared spectra in determination of calcium oxalate reveals both monohydrate and dehydrate forms. The techniques for precipitation in the specimen by reaction procedure methods also contributed in histological identification and confirmation for the presence of ergastic crystals [9].

#### **5. Economic importance of ergastic substances**

Ergastic crystals and related substances have well defined economic importance that includes protective, defensive and remedial properties. Applying ergastic substances in taxonomic consideration can be of considerable importance for review of existing taxonomic delimitation for clearer circumscription and evolutionary history of the taxa [12]. Diversity relationship of five genera in the family Polygonaceae based on ergastic evidences has been worked out by Conrad and Idu [13].

Inulin as a carbohydrate is considered indigestible, which necessitates extensive processing (i.e., roasting) prior to consumption, hence the above effect if unprocessed or form a large percentage of diet [14]. Tannins are usually non-bioavailable and like inulin show some degree of anti-nutritive properties as they can bind and precipitate proteins and carbohydrates [15].

Raphide crystals play a role in reducing metal toxicity. This suggestion has largely been based on the observation that such crystals can have many other divalents [16–18]. Quantity of oxalic acid content in plants is different in different parts i.e. in many cases rhizomes are observed to with higher content than in leaves or tender parts [19]. Oxaloacetic acid is component in functioning of guard cells in plants, which follow Hatch and Slack pathway Oxalates provide tolerance to aluminum toxicity. According to Rajendra and Shivay, oxalates have involvement in phytoremediation of soils rendered toxic by heavy metals like lead, cadmium and

zinc [20]. Oxalic acid is also reported to help in the accumulation of heavy metals, cadmium, nickel, zinc, etc. by hyper-accumulators that are being utilized in phytoremediation of soils affected by toxicity of these heavy metals [21–23].

Of the five types of calcium oxalate crystals, raphides are prominent ones in terms of size and quantity as it can occur intercellular and intracellular. Calcium oxalates gets incorporated in human body through plant-based food. These along with the endogenously synthesized content contribute to kidney problems. Studies reveal that calcium oxalates are present in algae, fungi and lichens in addition to their presence in higher plants. Out of all the three forms of calcium oxalate, the monohydrate form is the one widely reported to cause kidney problems [24].

Calcium oxalate, a potential causative agent of human kidney stones, can range from 3 to 80% of the dry weight of various plants [25, 26] and it can contribute up to 70 or 75% of the composition of kidney stones [27]. Deleterious influence of raphides includes promoting kidney stone formation, irritation to throat, mouth and skin [28–32]. Excess presence of raphides, in conjugation with cytotoxic compounds [5, 33], can render the food poisonous and is responsible for mentionable fatalities every year [34, 35].

Crystallized calcium oxalates that appear, as bundles of needles under light microscopes are usually raphides [28, 36]. It is believed that herbivory enhances raphide production in plant cells and the coexisting cysteine proteases together with other defensive chemicals promote protection against grazing animals. The needle like raphides cause bruising the alimentary tract lining of herbivores and also causes irritation due to presence of cysteine proteases [31]. The additive effect of irritants such as cysteine proteases and raphides has been proved in larvae and caterpillar [37].

## 6. Plant families and the types of ergastic crystals in plants

The distribution and characterization of ergastic crystals indicate that they are unique entities in the circumscription and delimitation of various taxa. A review of the calcium oxalate crystals in plants is presented in detail [30]. Calcium oxalate crystals are widely distributed and enlisted in 215 plant families [38]. Systematic significance of the formation, occurrence and distribution of crystals were studied in leaves of 22 species of *Combretum* [39]. Studies on anther anatomy of 167 species of Fabaceae plant family and wood anatomy of 139 species of Verbenaceae plant family reported several types of crystals [40, 41]. The wood anatomy of the plant family Lauraceae revealed the presence of significant prismatic crystals while the plant family Tiliaceae shows the presence of conglomerate crystals [42].

Christina reviewed the structure and systematics of calcium crystals in monocotyledons especially their occurrence of these crystal types, with respect to current systematics [43]. The three main types of calcium oxalate crystal that occur in monocotyledons are raphides, styloids and druses, although intermediates are sometimes recorded. It is inferred that the presence or absence of the different crystal types may represent ‘useful’ taxonomic characters. Further, styloids are characteristic of some families of Asparagales, notably Iridaceae, where



raphides are entirely absent. Raphides are predominant in Monocots mainly seen in leaf petiole of Araceae [42, 44] Styloids are seen in Agavaceae [45]. In *Dracaena sanderiana* (Liliaceae) two types of intracellular calcium oxalate deposits are reported: calcium oxalate monohydrate raphides and solitary calcium oxalate dihydrate crystals [46]. Archeological significance of raphides in Araceae is studied by [6].

In Gymnosperms, druses, prismatic crystals and solitary crystals are observed. Druses are seen in the leaf vascular tissue of *Ginkgo biloba* [47]. In Pinaceae, wood CaOx ray cells and cork of stem contain solitary and prismatic crystals. Calcium oxalate crystals are considered to enhance internal source of carbon dioxide in plants [48]. This is recorded in *Amaranthus hybridus* (Amaranthaceae), *Dianthus chinensis* (Caryophyllaceae), *Pelargonium peltatum* (Gesneriaceae) and *Portulacaria afra* (Portulacaceae). Occurrence, type and location of calcium oxalate crystals have been investigated in *Achyranthes aspera* (Amaranthaceae), *Adhatoda zeylanica* (Acanthaceae), *Aerva lanata* (Amaranthaceae), *Asparagus racemosus* (Asparagaceae), *Atalantia monophylla* (Rutaceae), *Bridelia crenulata* (Euphorbiaceae), *Carica papaya* (Caricaceae), *Carissa spinarum* (Apocynaceae), *Plumeria rubra* (Apocynaceae) *Monochoria vaginalis* (Pontederiaceae) [49].

The types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants have been studied. Crystal sands and prismatic crystals were of rare occurrences. Prismatic crystals were observed in the leaf mesophyll cells of *Nerium oleander* and *Cynanchum acutum*. It was concluded that there is no absolute correlation between the presence and type of calcium oxalate crystals and toxic plant organs.

An extensive enumeration of calcium oxalate crystal reports has been done [28] in 215 plant families including genus *Sida* of Malvaceae. Further, the relation between herbivory and calcium concentration has been recorded in the leaves of *Sida* species. Cell mediated crystallization of calcium oxalate is reported by Webb [25]. The structures of cystoliths in selected taxa of the genus *Ficus* L. (Moraceae) in the Malaysia Peninsular have been investigated [50]. The characteristics of the cystoliths may not suitably be used as a taxonomic marker but it can be useful as additional character for group identification in *Ficus*.

New and unusual forms of calcium oxalate raphide crystals in the plant kingdom [51] from the tubers of *Dioscorea polystachya*—six-sided needles with pointed ends and four-sided needles with beveled ends. The production of calcium oxalate crystals has a long evolutionary history and probably evolved independently in major clades of symbiotic fungi and several times in the plantae, as part of the overall process of bio-mineralization [29].

## 7. Genes that contributes in production of ergastic crystals

Even though the nature of control of crystal shape and composition phenomena is yet fully unknown the taxonomic value of crystal shape assumes that it is under genetic control. The scanty knowledge about the mechanisms regulating production and crystal formation is another reason to establish the genetic contribution. Leaves from a chemically mutagenized *Medicago truncatula* population were visually screened for alterations in calcium oxalate crystal

formation was performed by Nakata and Mc Conn and seven different classes of calcium oxalate defective mutants were identified. Genetic analysis suggested that crystal formation is a complex process involving more than seven loci [52]. Oxalate-producing plants, which include many crop plants, accumulate oxalate in the range of 3–80% (w/w) of their dry weight [25].

Of the several metabolic pathways proposed, cleavage of ascorbic acid appears to be the most appreciable [53]. According to this view, once produced the oxalate combines with calcium to generate variety of crystal shapes and sizes. Further studies are required to identify the pathway(s) of oxalate production and calcium oxalate crystal formation.

A genetic approach would circumvent such technical limitations (e.g. idioblast number) and is a proven complement of biochemical and cellular investigations. Although the specific genes that have been altered are not yet to be identified it is understood that the control of crystal morphology is complex and under strict genetic control. As suggested by studies in other systems, mutations affecting protein, lipid, or polysaccharide function could contribute to alterations in crystal size or shape. Roles in ion balance (e.g. calcium regulation), in tissue support, in plant defense, in light gathering and reflection, and in detoxification have all been proposed [30]. Calcium oxalate crystals rapidly increase in size and number as the concentration of calcium in the plant environment is increased [54].

Nutritional studies have shown that oxalate is an anti-nutrient that sequesters calcium in a state that renders it unavailable for nutritional absorption by humans. Even though increasing nutritional quality by biotechnological method is fast in progress attempts to reduce or nullify the amount or effect of potential anti-nutritional agents from the economically useful plants is important.

## 8. Ergastic crystals and medicinal raw drug identification

Correct taxonomic identification of plants is most important before proceeding to any analytical procedure and utilization. Comparative approach on morphological and anatomical features provides distinguishable features for species to species, which is well established in identification of some medicinally useful plants [19]. Morphological features of vegetative parts with qualitative value vary with respect to habitat change and growing regions when cultivars are considered. As flowers fruits and seeds are produced seasonally and when the economically important part is leaves rhizome, corm or tuber identification based on reliable anatomical characteristics may be useful for making differentiation. Ergastic crystals can serve as an important diagnostic tool for the identification of economically important species. Presence of characteristic cuboidal ergastic crystal in the leaves of several plant species including *Costus speciosus* has been well reported [1, 55]. Cuboidal crystals of calcium oxalate are present in the mesophyll cells of *Costus speciosus* and are not reported in mesophyll cell of *Costus pictus* leaves, it can become a consistent and easily identifiable characteristic between these two species. Calcium oxalate crystal is smaller in size towards the tip of the aerial shoot in *Costus pictus* but bigger towards the base of the aerial stem. The crystal size in underground rhizome was found comparatively bigger than those in aerial

shoot [19]. So the presence of ergastic crystals from various plant parts, its size and structure is an important taxonomic key for the making difference between medicinally important species *Costus pictus* and *Costus speciosus*.

## 9. Conclusion

Land resources are blessed with numerable plants, which are of multifarious use. The combined effect of plant introduction and cultivation has largely accelerated the interest of scientists and industrialists to focus on herbal medicine and other economic products. For the sake of consumption of various plants with diverse phyto combinations processing of various level is suggestive. Even though modern biotechnological methods for analyzing and ensuring standards for stabilizing ergastic crystal concentration in raw, prepared food and herbal medicine is not available; traditional methods such as heating, boiling, frying, baking, battering, mashing, fermentation and sun drying, likely work by neutralization of cysteine proteases or through release of raphides from idioblasts or both. Neutralization of calcium oxalate from the dietary compounds still remains a bigger health question than the neutralization of specific crystal form of raphides. A traditional approach of avoiding plant pericarp rich in calcium oxalate and multilayered skin with lignified walls has beneficial effects. Discovery of fungi and bacteria that can break down calcium oxalate and plant genes that regulate calcium oxalate formation and crystallization have offered hope to counteract calcium oxalate toxicity.

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