Ergastic Crystals in Lettuce Tree: A Potential Antinutrient

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ABSTRACT
Plants are known for enriching human life with nutrition, but many plant parts have ergastic crystals in the form of calcium oxalate crystals. The dietary ergastic crystals are believed to be anti–nutrients as they accumulate in many medical cases and form complexes. Several of the medicinally useful plants contain these crystals and consumption of such plant materials afresh can cause health problems in humans. Present study focuses on the anti-nutrient component in the form of ergastic crystals, which is present in its vegetative parts of *Pisonia alba*.
Pisonia alba commonly known as lettuce tree or cabbage tree belonging to Nyctaginaceae family is widely used as a leafy vegetable and medicine in South India. Calcium oxalate crystals are abundant in its leaf, stem and petiole as raphide bundles. On an average size of raphide bundles ranges from 0.009mm-0.012mm in length and 0.008mm-0.011mm in thickness. Individual crystals are pointed on both ends, needle like and transparent with size ranging from 0.006mm-0.011mm in length. L.S of stem and petiole reveal raphide bundles arranged parallel to the long axis, distributed in cortex and stele. In leaves raphide bundles show a transverse arrangement on the lamina interspersed with the photosynthetic tissue. Older leaves exhibit elevated number of raphide bundles than younger leaves. Length, breadth and fresh weight of the individual leaf ranges from 19-26 cm, 11-18 cm and 0.003-0.008 gm respectively. Area of lamina ranges from 150-400 cm^2. The crystals are persistent in the material even after cooking and processing like stir-frying in oil, blanching, and pickling in apple cider vinegar. Therefore, careful evaluation of traditional and ethno-botanical knowledge is required before establishing plants and their parts as vegetable, as some of them may have deleterious phyto-constituents that has elevated potential to cause renal defects.

KEYWORDS
cooking, deleterious, establishing

1. Introduction

Among the vast combination of phyto-chemicals that are useful for the consumer world there are certain deleterious chemicals or its combinations. The undesirable component in phytochemicals to the consumers requires to be eliminated and therefore knowledge of ergastic bodies of Calcium oxalate called ergastic crystals have been reported from all the five kingdoms of organisms namely monera, protista, fungi, plantae and animalia (Nakata, 2003). Presence of ergastic crystals have been observed in most organs and tissues such as leaf, stem, flower, fruit, seed, root and bark and they exist in different sizes and shapes (Lersten and Horner, 2008).
Druses, styloids, raphides, prisms and crystal sands are the most common morphologies of calcium oxalate crystals found in plants (Franceschi and Horner, 1980). Crystals of calcium oxalate and calcium carbonate are found in plants. Evans (1996) estimated that 1-20 % dry weight of a plant can be accounted to calcium oxalate crystals. The morphology and size acquired by the crystal are related to the size and shape of the cells in which they are formed (Scurfield et al., 1973). Mc Nair (1932) has listed out 215 families of plants with at least some species containing calcium oxalate crystals.

Extensive studies on the taxonomic significance of ergastic crystals in various families such as Verbenaceae (Mathew and Shan, 1984), Dioscoreaceae (Okoli and Green, 1987), Cucurbitaceae (Okoli, 1988), Liliaceae (Tilton, 1978), Agavaceae (Arnott et al., 1965) have been done.

Ergastic crystals accumulate in specialized cells called crystal idioblasts. The number and location of crystal idioblasts vary among taxa (Nakata and Mc Conn 2000). The functions of ergastic crystals in plants have been uncertain but various properties have been assigned to them such as calcium regulation in plant cells (Franceschi and Nakata, 2005), protection against herbivores (Flores, 2001), detoxification of heavy metals, light gathering and reflection of light (Franceschi et al., 2007). Animals foraging on calcium oxalate rich plants such as *Amaranthus* and *Chenopodium* have been reported to develop peri renal edema in kidney tubules (Buck et al., 1966; Marshall et al., 1967).

Pisonia alba commonly known as lettuce tree or cabbage tree belonging to Nyctaginaceae family is widely used as a leafy vegetable and medicine in South India. It is also commonly used as a fodder plant. Ethnobotanically *Pisonia alba* is widely used as an anti-inflammatory, antidiabetic and antifilariatic (Kirtikar and Basu, 1935). The plant is also an ornamental grown for its handsome light green foliage. *Pisonia alba* grows to a large shrub or even a tree with inconspicuous green flowers and sticky seeds contributing to the ornithophilous mode of seed dissemination. They were natives of Seychelles and Andamans islands and spread to land masses by migratory birds (Komdeur and Kats, 1999).

2. Materials And Methods

Vegetative parts of *Pisonia alba* were collected from two localities of peninsular India. One from the western coast and the other from the eastern coast. Data on morphological characters were recorded from the field and further processing and extensive study for ergastic crystal analysis was conducted in the laboratory. Anatomic evaluation of vegetative parts was performed under Carl Zeiss Primostar microscope for the study of calcium oxalate crystals. Microscopic photographs were taken in Axiocam. Care was taken in transporting the material afresh.

Persistence of calcium oxalate crystals in samples were tested by heating in coconut oil and apple cider vinegar. Ash test was also performed.

3. Results

Leaves of *Pisonia alba* are simple, petiolate with bright green coloration shown in Figure 1 (a) and (b). It measures from Length, breadth and fresh weight of the individual leaf ranges from 19-26 cm, 11-18 cm and 0.003-0.008 gm respectively. Area of lamina ranges from 150-400 cm^2.

Microscopic evaluation of *Pisonia alba* leaf peels exhibit the presence of needle shaped calcium oxalate crystals in bundles (Fig. 2). The barrel shaped raphide bundles on disturbance were observed shattered to numerous transparent needle crystals pointed on both ends. The length of individual raphide bundles ranges from 0.009-0.012 mm and width from 0.08-0.011 mm. Individual crystals were seen in various lengths ranging from 0.06-0.011 mm. The raphide bundles are seen interspersed with the photosynthetic tissue (Fig. 3).

Longitudinal sections of *Pisonia alba* stem reveals raphide bundles arranged parallel to the long axis. Crystals observed in cortex, pith and vascular bundles. Numerous raphide bundles are seen in close vicinity to vascular bundles in the stem. Arrangement and distribution of raphide crystals in petiole is similar to that of stem.

Heating the leef peels of *Pisonia alba* in apple cider vinegar doesnot show any change in the raphide bundle number, shape and dimension (Fig. 5). Mesophyll tissue colour was altered from bright green to dull brown on reaction with cider vinegar. Effect of leef peels on heating with coconut oil also

exhibited no change in the crystal size, shape or dimension (Fig. 4) Ash test on leaf sample of *Pisonia alba* confirm the presence of raphide bundles intact in ash. No alterations in dimension of crystals were noted.Shape was retained and transparent nature was lost.

4. Discussions

Calcium oxalate crystals in plants have significance in modern diet as high oxalate levels in certain plant-derived foods can cause serious health problems (Nguyên and Savage, 2013). Until recently it was believed that dietary calcium oxalate has very little contribution to kidney stone formation. But it was experimentally proven that dietary oxalate contributes to 24-53% of urinary oxalate from an intake of 10 to 250 mg oxalate per day (Holmes et al., 2001). Dietary oxalate was found out to be an important factor in nephrolithiasis or kidney stone formation (Holmes and Assimos, 2004). It was also found out that calcium and oxalate have an equal contribution towards the precipitation of calcium oxalate crystals in urine (Pak et al., 2004).

When oxalate-rich foods, such as spinach or rhubarb, are consumed, daily intake may even exceed 1000 mg/day (Hoppe et al., 2005). Seasonal rural diets of India even raise the values up to 2000mg/day (Siener et al., 2003).

In the present study, the calcium oxalate load per area is noted highest in mature leaves (6.8 bundles/0.009mm^2), moderate in stem and petiole and lowest in young leaves. Samples from the western coast exhibited longer raphide bundles in stem than in mature leaves, (2.5 bundles/0.009mm^2 stem and petiole from eastern coast. A prominent increase in raphide bundle length was observed in mature leaf samples of western coast (0.011mm) than from eastern coast.

Raphide bundle width was shortest in the young leaf samples of east coast with an average of 0.0081mm *Pisonia alba* as a leafy vegetable, fodder plant and ethnobotanic remedy for various ailments in South India, needs special mention as it has elevated calcium oxalate load in stem, leaves and petiole. Careful evaluation needed before utilizing plant as it has potential for causing renal ailments.

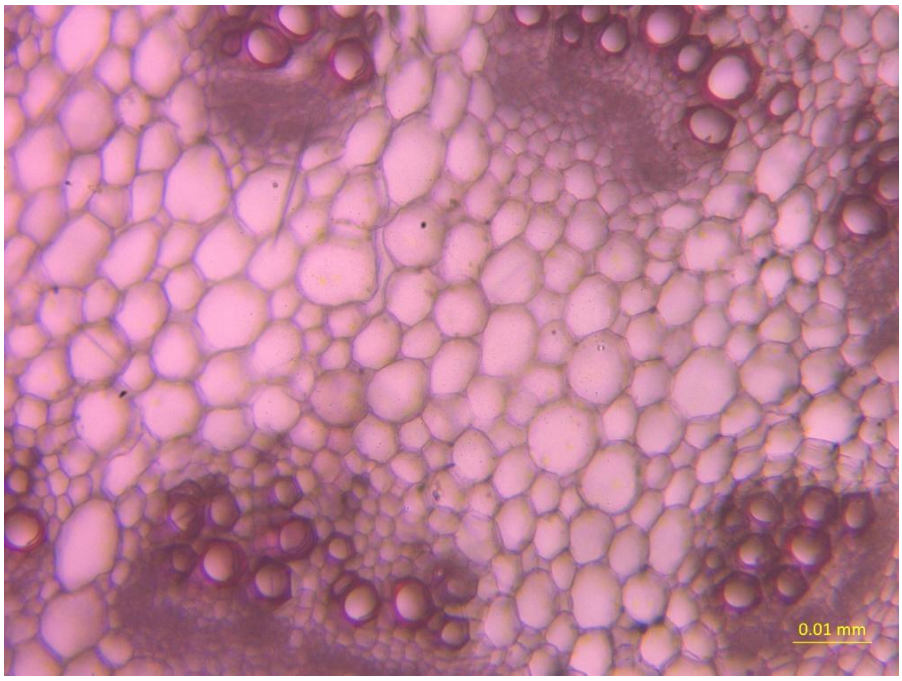

Figure 1

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(a) (b)

Figure 2

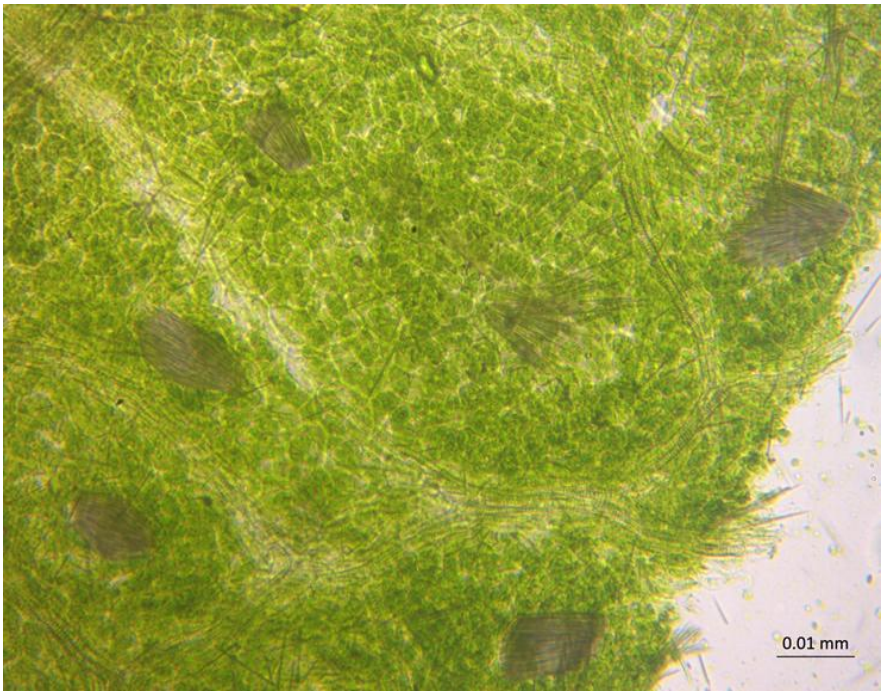

Figure 3

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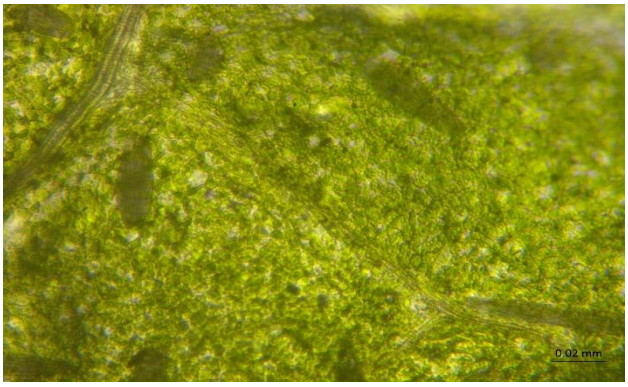

Figure 4

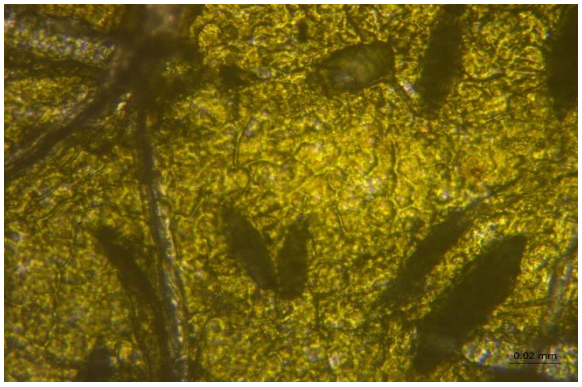

Figure 5

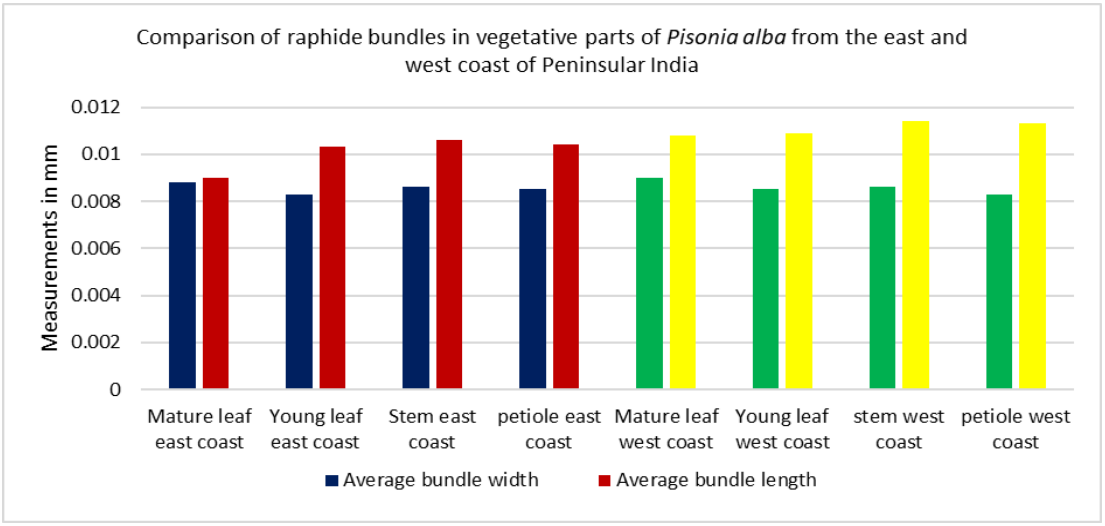

Figure 6 Comparison of Raphide bundles in vegetative parts of *Pisonia alba* from the East and West Coast of Peninsular India

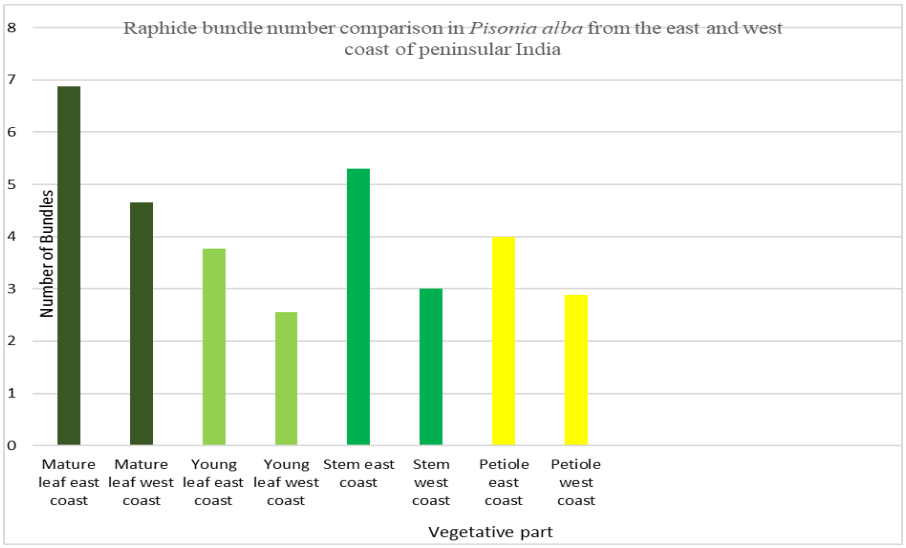

Figure 7 Raphide bundle number comparison in *Pisonia alba* from the East and West Coast of Peninsular India

5. CONCLUSION

Pisonia alba exhibit calcium oxalate raphide bundles in leaves, stem and petiole. Leaf samples collected from the east coast exhibit an elevated number of raphide bundles per area when compared to that from the west coast. The number of raphide bundles per area in young leaves, stem, and petiole samples also stay higher in the east coast samples than the west coast ones. Calcium oxalate load is maximum in mature leaves and minimum in younger leaves. Stem and petiole samples show a moderate calcium oxalate load compared to mature leaves. Cooking procedures like boiling in water, stir frying in oil, pickling in vinegar and dry roasting has no visible effect in the calcium oxalate load of *Pisonia alba.* This points to the persistence of crystals in food even after cooking. Calcium oxalate in diet has established influence on renal ailments as urolithiasis leading to renal failure in acute cases. The study points to the antinutrient effect of calcium oxalate raphide crystals in *Pisonia alba*, and hence the ethnobotanical and dietary and medical uses of it should be carefully evaluated.

REFERENCES

[1] Arnott, H. J. ,Pantard F. G. E. and Steinflink, H. 1965. Structure of calcium oxalate monohydrate. Nature **208** : 1197-1198

[2] Buck, W. B., Preston K.S., Abel M. and Marshall. 1966. Peri renal edema in swine: a disease caused by common weeds. J.Am. Vet. Med.Assoc. **148** :1525-1531

[3] Evans, W.C. *Trease and Evan's Pharmacognosy* (14th Ed.) W B Saunders Co.Ltd. , London (1996).

[4] Flores, B.2001. Herbivory and Calcium Concentration Affect Calcium Oxalate Crystal formation in Leaves of Sida (Malvaceae).*Annals of Botany,* **88**, 387-391 .

[5] Franceschi, V.R &Hornes, H.T. 1980. Calcium oxalate crystals in plants.*Bot. Rev.***46**, 361-427.

[6] Franceschi, V.R. and Nakata, P.A. 2005. Calcium Oxalate in plants: Formation and function *Annual Review of Plant Biology,***5** 641-671 .

[7] Holmes, R. P., Assimos, D. G. The impact of dietary oxalate on kidney stone formation. *Urol Res*. 2004; 32: 311-316.

[8] Holmes, R. P., Goodman, H. O., Assimos, D. G. Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int*. 2001; 59:270-276.

[9] Hoppe, B., vonUnruh, G.E., Laube, N. Oxalate degrading bacteria: new treatment option for patients with primary and secondary hyperoxaluria? Urol Res 2005; 33: 372–375.

[10] Justin R Nayagam. 2015. Ergastic Crystals In Identification Of *Costus pictus*: A Medicinal Spiral Ginger. JEBAS **3**(4) 378-383.

[11] Kiritikar, K. and Basu, L. 1935. Indian Medicinal Plants, Allahabad, India, Vol. III, 2nd ed., pp: 1817-1818.

[12] Komdeur, J. Kats, R. K. 1999. Predation risk affects trade-off between nest guarding and foraging in Seychelles warblers. Behav Ecol :**10**(6):648-58.

[13] Kuo-Huang, L.L., Ku, M.S.B.,Franceschi,V.R. 2007. Correlations between Calcium Oxalate Crystals and Photosynthetic Activities in Palisade Cells of Shade Adapted *Peperomia glabella,*,Botany **48**, 155-164 .

[14] Lersten, N. R. & Horner, M.T. 2008. Crystal macro patterns in leaves of Fagaceae and Nothofagaceae: a comparative study. *Plant systs. Evol.* **271**, 239-253.

[15] Mathew, L., and Shan, G.L. 1984. Crystals and their Taxonomic significance in some Verbenaceae.*Bot. J. Linn Soc*., **88**, 279-289 .

[16] Mc Nair, J. B. 1932. The interrelation between substances in plants: essential oils and resins, cyanogen and oxalate. Am. J. Bot. **19** :255-271

[17] Nakata, P.A. 2003. Advances in our understanding of calcium oxalate crystal formation and function in plants.*Plant Sci.* **164**, 901-909.

[18] Nakata, P.A. and Mc Conn, M.M. 2000. Isolation of *Medicago truncatula* mutants defective in calcium oxalate crystal formation. *Plant Physiology,* **124,** 1097-1104 .

[19] Nguyen, H.V.H., Savage, G.P. Oxalate content of New Zealand grown and imported fruits. J. Food Compos. Anal.2013; 31, 180–184.

[20] Okoli, B.E. and Green, B.O.1987. Histochemical localization of Calcium oxalate crystals in Starch grains of gums (Dioscorea L.) *Ann. Bot.***60**: 391-394 .

[21] Okoli, B.E.1988. On the probable function and taxonomic value of calcium oxalate crystals in Cucurbitaceae. *Fedes Report*. **99**: 139-142.

[22] Pak, C.Y.C, Adams, Huet B., Poindexter, J.R., Pearle, M.S., Peterson, R.D., Moe, O.W. Relative effect of urinary calcium and oxalate on saturation of calcium oxalate. *Kidney Int*. 2004; 66: 2032-2037.

[23] Scurfield, G., Michell, A.J.,Silva, S.R. 1973. Crystals in woody stems. *Bot. J. Linn Soc*., 66 (4), 277-289.

[24] Siener, R., Ebert, D., Nicolay, C. Dietary risk factors for hyperoxaluria in calcium oxalate stone formers. Kidney Int. 2003; 63: 1037–1043

[25] Tilton, V. R. 1978. A developmental and histochemical study of the female reproductive system in *Ornithogalum caudatum* Ait. (Liliaceae) using ligh tand electron microscopy. Ph.D. Dissertation, Iowa State University, Ames.