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SPATIAL VARIATION OF OXALATE, PHENOLIC CONTENT, AND CALCIUM OXALATE CRYSTAL COVERAGE IN SELECTED ROOT VEGETABLES

Renu Rajan[1], **Justin R Nayagam**[2*]

[1,2] Department of Botany, Union Christian College, Aluva-683102, Kerala, India.
Corresponding Author: Justin R Nayagam, justinr@uccollege.edu.in

ABSTRACT

Oxalates are naturally occurring antinutritional compounds in root vegetables, existing in both soluble and insoluble forms, often deposited as calcium oxalate crystals. The present study evaluated the tissue-specific distribution of total oxalate, total phenolic content, and calcium oxalate crystal morphology in selected root vegetables—*Manihot esculenta*, *Ipomoea batatas*, *Raphanus sativus*, *Beta vulgaris*, and *Daucus carota*. Total oxalate content was quantified using permanganometric titration and spectrophotometric methods, while total phenolic content was estimated using standard colorimetric assays. Crystal morphology and the area occupied by calcium oxalate crystals in the outer skin, middle cortex, and inner pith were analyzed using ZEN 4 and ImageJ software. Crystal sand was the predominant crystal type observed, with druse crystals restricted to *Ipomoea batatas*. The study highlights differential oxalate partitioning and crystal deposition patterns within root tissues, providing insights into oxalate bioavailability, plant physiological regulation, and dietary implications for human consumption.

KEYWORDS: Oxalates, Calcium oxalate, Root tubers, Phenolics, Crystal deposition, Bioavailability

INTRODUCTION

Root vegetables constitute an important component of human diets worldwide, providing carbohydrates, minerals, dietary fiber, and bioactive compounds. Alongside their nutritional value, many root vegetables accumulate oxalates, which occur in plants as soluble oxalic acid and insoluble calcium oxalate salts. Dietary oxalates are of clinical relevance, as excessive intake of soluble oxalate can chelate dietary calcium, reduce mineral bioavailability, and contribute to the formation of calcium oxalate kidney stones, which account for nearly 70–80% of renal calculi cases globally[1].

Calcium oxalate crystal formation in plants is a genetically and physiologically regulated process, occurring predominantly within specialized idioblast cells. These crystals serve multiple functions, including calcium homeostasis, detoxification of excess calcium, tissue strengthening, and defense against herbivory[2]. Calcium oxalate crystals occur in distinct morphologies such as crystal sand, druses, raphides, and styloids, many of which are taxonomically informative and tissue-specific[3]. Despite extensive anatomical documentation of crystal morphology in leaves and stems, comparable studies in edible root vegetables remain limited.

Recent research has demonstrated that oxalate synthesis and crystal deposition are highly responsive to environmental factors such as soil calcium availability, nitrate concentration, water stress, and climatic conditions, leading to significant regional variation in oxalate content within the same crop species[4]. This variability underscores the need for localized oxalate profiling of commonly consumed vegetables, particularly those forming dietary staples.

Phenolic compounds represent another major class of plant secondary metabolites with antioxidant, anti-inflammatory, and potential pharmacological properties. Their accumulation in root tissues is influenced by developmental stage and environmental stress, and emerging evidence suggests an interaction between phenolic metabolism and oxalate regulation in plants[5]. However, tissue-specific distribution of phenolics in relation to oxalate load and calcium oxalate crystal deposition in root vegetables has not been comprehensively evaluated.

Given the nutritional, physiological, and health implications of oxalates and phenolic compounds, the present study integrates biochemical estimation and image-based analysis to comparatively assess total oxalate content, total phenolic content, calcium oxalate crystal morphology, and crystal area distribution across different tissue layers of selected root vegetables.

MATERIALS AND METHODS

Plant Material

Fresh, healthy root vegetables—*Manihot esculenta*, *Ipomoea batatas*, *Raphanus sativus*, *Beta vulgaris*, and *Daucus carota*—were procured from local markets. Roots were thoroughly washed under running tap water to remove soil and debris and subsequently rinsed with distilled water. Each root was sectioned into three distinct tissue regions: outer skin (O), middle cortex (M), and inner pith (I).

Estimation of Total Oxalate Content

Total oxalate content was determined using both permanganometric titration and spectrophotometric methods to ensure analytical reliability. Tissue samples were oven-dried, powdered, and extracted following standard protocols. Oxalate content was expressed as mg/100 g dry weight. Results from both methods were compared to validate consistency.

Estimation of Total Phenolic Content

Total phenolic content was estimated using a colorimetric method and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight. Extracts from each tissue layer were analyzed in triplicate to ensure reproducibility.

Microscopic Analysis and Crystal Morphology

Thin transverse sections from each tissue layer were prepared and examined under a light microscope to identify calcium oxalate crystal types. Crystal morphology was categorized based on established anatomical descriptions.

Quantification of Crystal Area

High-resolution microscopic images were captured using ZEN 4 imaging software. The area occupied by calcium oxalate crystals was quantified using ImageJ software and expressed as µm²/cm². Measurements were taken from multiple fields per section to minimize sampling error.

Statistical Analysis

All experiments were conducted in triplicate. Data were statistically analyzed using one-way ANOVA at a 95% confidence level to assess variation within and between tissue layers using SPSS software. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Selected root vegetables were chemically evaluated using permanganometry and spectrophotometry for total oxalate content to quantify oxalate content in their outer skin, middle cortex and inner pith of them. Total phenol content of the same parts of plants were evaluated for quantifying phenol in them. Area occupied by crystals in the same parts of the selected plants were conducted using ZEN 4 and Image J software to physically analyse the area under crystal cover.

In the root vegetables studied crystal sand was the most common type of crystal observed. *Manihot esculenta*, *Beta vulgaris, Raphanus sativus* and *Daucus carota* exhibited crystal sands in their roots and styloids in *Ipomoea batatas.* Crystal morphology (Table1) is an identification character in taxonomy and root vegetables are very scantily documented for crystal morphology. Crystal morphology of root vegetables such as *Beta vulgaris* and *Ipomoea batatas*[25] was in accordance with previous morphological studies

Table 1. Types of Calcium oxalate Crystals in Root Tubers

SI No:	Plant Studied	Family	CaOx crystal present
1	*Manihot esculenta*	Euphorbiaceae	Crystal sand
2	*Ipomoea batatas*	Convolvulaceae	Druse
3	*Raphanus sativus*	Brassicaceae	Crystal sand
4	*Beta vulgaris*	Amaranthaceae	Crystal sand
5	*Daucus carota*	Apiaceae	Crystal sand

Total Oxalate (TO) Content in Root Vegetables

Highest TO was observed in outer skin of *Daucus carota* which accommodated 1.055 mg/g expressed as 105.5mg/100g TO which is very high than permissible level TO (50mg/100). Least TO was observed in outer skin of *Beta vulgaris* (26.7mg/100g) which is within the permissible range of total oxalate. *Ipomoea batatas* (77.4mg/100g) and *Raphanus sativus* (80.6mg/100g) showed higher than permissible level of TO in the outer skin. Manihot esculenta expressed 59 mg/100g TO which is slightly above permissible range but lower when compared to outer skins of *Ipomoea batatas, Raphanus sativus* and *Daucus carota.* Previous studies on skin oxalate analysis are not available for comparison. Comparison of total oxalate in middle cortex of root vegetables showed that both permanganometric TO and spectrophotometric TO values were comparable. Highest TO was in middle cortex of *Daucus carota* (105.4mg/100g) which was twice the permissible TO level. Least TO was in *Beta vulgaris* (26.6mg/100g) which was within the TO permissible level. *Ipomoea batatas*(76mg/100g) and *Raphanus sativus* (80.3mg/100g) showed higher than permissible level of TO in the middle cortex. *Manihot esculenta* expressed (56.5 mg/100g) which is slightly above permissible range but lower when compared to middle cortex of *Ipomoea batatas, Raphanus sativus* and *Daucus carota.* Comparison of total oxalate in inner pith (I) of root vegetables showed that both permanganometric TO and spectrophotometric TO values were comparable. Highest TO was in inner pith of *Daucus carota* (105.3mg/100g) which was twice the permissible TO level. Least TO was in *Beta vulgaris* (26.4mg/100g) which was within the TO permissible level. *Ipomoea batatas* (77.6mg/100g) and *Raphanus sativus* (80.2mg/100g) showed higher than permissible level of TO in the inner pith. *Manihot esculenta* expressed (58.5 mg/100g) TO which is slightly above permissible range but lower when compared to inner pith of *Ipomoea batatas, Raphanus sativus* and *Daucus carota.* Out of the root vegetables studied, *Daucus carota* showed highest Total oxalate load. Only *Beta vulgaris* has TO below permissible range.

Table 2. Comparison of total oxalate content in different root tubers by permanganometry and spectrophotometry

SI	Plant Studied	TO(Permanganometry)	TO(Spectrophotometry)
		Mean±SD	Mean±SD

No:		**O**	**M**	**I**	**O**	**M**	**I**
1	*Manihot esculenta*	0.590±0.005^{a}	0.565±0.007^{b}	0.585±0.003^{a}	0.590±0.002^{a}	0.567±0.005^{c}	0.582±0.006^{b}
2	*Ipomoea batatas*	0.774±0.002^{a}	0.760±0.005^{b}	0.776±0.003^{a}	0.774±0.008^{a}	0.760±0.008^{b}	0.775±0.008^{a}
3	*Raphanus sativus*	0.806±0.001^{a}	0.803±0.001^{b}	0.802±0.001^{b}	0.805±0.008^{a}	0.804±0.007^{a}	0.803±0.008^{a}
4	*Beta vulgaris*	0.267±0.001^{a}	0.266±0.001^{a}	0.264±0.001^{b}	0.267±0.008^{a}	0.265±0.008^{a}	0.264±0.008^{a}
5	*Daucus carota*	1.055±0.001^{a}	1.054±0.002^{a}	1.053±0.001^{a}	1.050±0.008^{a}	1.060±0.006^{a}	1.053±0.008^{a}

Table 3. Comparison of Total Phenol (TP) Content in Root tubers

SI	**Plant Studied**	**TP**		
		Mean±SD		
No:		**O**	**M**	**I**
1	*Manihot esculenta*	0.246±0.001^{a}	0.228±0.003^{b}	0.247±0.002^{a}
2	*Ipomoea batatas*	0.390±0.002^{a}	0.355±0.003^{b}	0.386±0.003^{a}
3	*Raphanus sativus*	0.402±0.006^{a}	0.336±0.002^{b}	0.397±0.003^{a}
4	*Beta vulgaris*	0.474±0.002^{a}	0.457±0.005^{b}	0.476±0.002^{a}
5	*Daucus carota*	0.442±0.001^{a}	0.436±0.003^{b}	0.440±0.002^{a}

Comparison of total phenol (TP) content of outer skin (O) (Table3) of root vegetables selected for study revealed that highest concentration of TP was in outer skin of *Beta vulgaris* (47.4 mg GAE/100g) followed by *Daucus carota* (44.2 mg GAE/100g). Similar range of TP was in *Ipomoea batatas (*39 mg GAE/100g) and *Raphanus sativus (*40.2 mg GAE/100g). Least TP was expressed by *Manihot esculenta* (24.6 mg GAE/100g) (4.2.1.5). Comparison of total phenol (TP) content of middle cortex (M) of root vegetables selected for study revealed that highest concentration of TP was in middle cortex of *Beta vulgaris* (33.6 mg GAE/100g) followed by *Daucus carota* (43.6 mg GAE/100g). Similar range of TP was in *Ipomoea batatas* (35.5 mg GAE/100g) and *Raphanus sativus* (33.6 mg GAE/100g). Least TP was expressed by *Manihot esculenta* (22.8 mg GAE/100g). Total phenol content in sweet potato. Comparison of total phenol (TP) content of inner pith (I) of root vegetables selected for study revealed that highest concentration of TP was in inner pith of *Beta vulgaris* (47.6 mg GAE/100g) followed by *Daucus carota* (44 mg GAE/100g). Similar range of TP was in *Ipomoea batatas (*38.6 mg GAE/100g) and *Raphanus sativus* (39.7 mg GAE/100g). Least TP was expressed by *Manihot esculenta* (24.7 mg GAE/100g).

Middle cortex of root vegetables showed reduced phenol content than outer skin and inner pith. But the difference was not statistically significant. Even then it would be better to exclude the outer skin from root vegetables before consumption by renal patients.

Table 4. Comparison of Crystal Area in Root Tubers

SI	**Plant Studied**	**Image Analysis**		
		Mean±SD		
No:		**O**	**M**	**I**
1	*Manihot esculenta*	5.523±0.38^{a}	4.426±0.16^{b}	4.811±0.13^{b}
2	*Ipomoea batatas*	3.334±0.18^{a}	2.218±0.13^{b}	2.228±0.07^{b}
3	*Raphanus sativus*	3.637±0.04^{a}	3.115±0.01^{b}	3.583±0.05^{a}
4	*Beta vulgaris*	4.664±0.13^{a}	4.056±0.06^{b}	4.651±0.05^{a}
5	*Daucus carota*	4.243±0.11^{a}	3.828±0.12^{b}	4.097±0.07^{a}

Area occupied by crystals in the outer skin (O), middle cortex (M) and inner pith (I) of root vegetables was compared using and to find out the distribution of calcium oxalate crystals in the different tissue layers of the root vegetable (Table 4). Comparison of crystal area occupied in outer skin (O) of root vegetables studied showed that maximum area occupied by crystals were in *Manihot esculenta* (5.523 µm^2/cm^2) followed by Beta vulgaris (4.664 µm^2/cm^2). Least area occupied by crystals were found in *Ipomoea batatas* (3.334 µm^2/cm^2). *Raphanus sativus* showed lesser area occupied by crystals (3.637 µm^2/cm^2) than *Daucus carota* (4.243 µm^2/cm^2). Comparison of crystal area occupied in middle cortex (M) of root vegetables studied showed that maximum area occupied by crystals were in *Manihot esculenta* (4.426 µm^2/cm^2) followed by Beta vulgaris (4.056 µm^2/cm^2). Least area occupied by crystals were found in *Ipomoea batatas* (2.218 µm^2/cm^2). *Raphanus sativus* showed lesser area occupied by crystals (3.115 µm^2/cm^2) than *Daucus carota* (3.828 µm^2/cm^2).

Comparison of crystal area occupied in inner pith (I) of root vegetables studied showed that maximum area occupied by crystals were in *Manihot esculenta* (4.811 µm^2/cm^2) followed by Beta vulgaris (4.651µm^2/cm^2). Least area occupied by crystals were found in *Ipomoea batatas* (2.228 µm^2/cm^2). *Raphanus sativus* showed lesser area occupied by crystals (3.583 µm^2/cm^2) than *Daucus carota* (4.097 µm^2/cm^2)

Outer skin of roots showed an increase in area occupied by crystals than middle cortex and inner pith. Manihot esculenta showed maximum area occupied by crystal in roots studied but TO load was lower in it, which points to the very low soluble oxalate content of *Manihot esculenta.* Large area covered by crystals were noted in *Beta vulgaris* also. But TO load was very less in it which again pointed to the presence of low soluble oxalate content and high insoluble oxalate content of the plant.

DISCUSSION

Calcium oxalate crystals and associated biochemical attributes, including total oxalate (TO) and total phenolic content (TP), varied significantly among the root vegetables studied, reflecting tissue-specific physiological differences, genetic factors, and environmental influences. Crystal sand was the predominant form in *Manihot esculenta*, *Beta vulgaris*, *Raphanus sativus*, and *Daucus carota*, whereas styloid crystals were characteristic of *Ipomoea batatas*. Crystal morphology has long been recognized as a taxonomic and physiological marker in plants[6], and recent microscopy studies confirm diverse crystal architectures and their roles in stress adaptation and nutrient regulation[7,12,21].

Comparison of TO (Fig 1) in the outer skin of root vegetables studied showed whether TO levels[26] are above or below permissible range which is 50mg/100g. Honow and Hesse[11] estimated total oxalate in *Daucus carota* (17.8 mg/100g), *Beta vulgaris* (36.9mg/100g), *Raphanus sativus* (11.2mg/100g), and Ogawa et al. [27]estimated 48.5mg/100g TO in *Daucus carota* which is not in accordance with the present study. It may be due to variation in climatic (Kuo-Huang et al., 2007) or environmental conditions (Xiaofeng et al., 2018) where the studies were conducted. Abdel- Moemin (2014) estimated total oxalate in sweet potato as 52mg/100g but was estimated higher in present study (76mg/100) as total oxalate varies with cultivar and environment differences. TO content did not differ significantly among the outer skin, middle cortex, and inner pith ($p > 0.05$), although interspecific differences were evident. *Daucus carota* consistently exhibited the highest TO, exceeding dietary limits[8], while *Beta vulgaris* remained within safe ranges. Elevated TO values relative to earlier studies highlight the strong influence of genotype, cultivation conditions, and local environmental factors[9,10,11].

Recent research demonstrates that nutrient availability, especially nitrogen, modulates oxalate biosynthesis and calcium partitioning in plants[13,22]. Moreover, processing and cooking duration have been shown to alter calcium oxalate crystallinity, influencing dietary oxalate load[9,23]. Studies on total oxalate estimation by Hesse and Honow[11] reported more than 50% change in total oxalate content on various food crops previously estimated by Ogawa and Takahashi[27]. This stresses the need for regional or local databases for oxalate content in all vegetables, fruits and other food crops, as the oxalate load in plants is dependent on climatic conditions[4] factors or soil parameters such as concentration of nitrates and soil ammonia[28] and water availability[29].

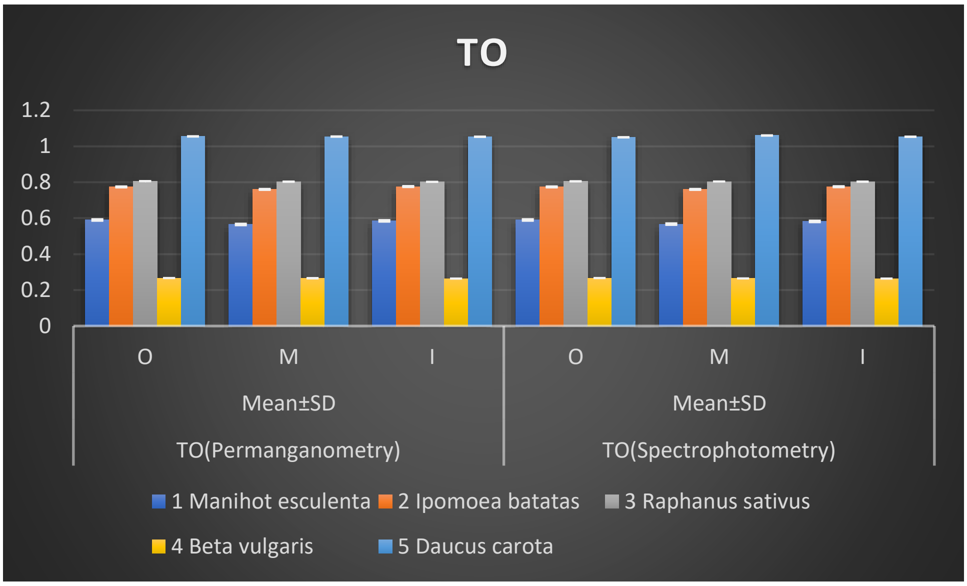

Figure 1: TO in Tubers

TP content (Fig 2) did not show statistically significant variation among tissue layers; however, *Beta vulgaris* consistently exhibited the highest phenolic content, supporting antioxidant potential. Phenolic variation is influenced by climatic, cultivar, and post-harvest factors[14,15,16]. Crystal area distribution, in contrast, varied significantly among tissue layers ($p < 0.05$), with the outer skin showing the highest density. The inverse relationship between crystal area and TO in *Manihot esculenta* and *Beta vulgaris* suggests predominance of insoluble oxalate, which reduces soluble oxalate toxicity[11,12,17]. Bioactive compounds such as polyphenols and saponins may further inhibit calcium oxalate crystallization, enhancing the nutritional safety of root vegetables[18,19,20]. Total phenol content in vegetables vary depending on the climatic factors, stage of ripeness, geographical distribution and storage practices[17,18]. Antioxidant activity in root tubers of India studied by Sreeramulu and Reghunath[19] also pointed to the difference in total phenol content of vegetables grown in different regions and climatically varied regions. Studies on alterations in temperature of root zone in hydroponically cultivated carrots resulted in positive correlation of root zone temperature to phenol content in the inner pith of root tubers[5]. In Raphanus sativus total phenol was estimated to be 11.85 mg GAE/100g[13] which is lower than what observed in the present study (39.7 mg GAE/100g) may be due to climatic factor differences and temperature difference in the regions the root tubers are grown.

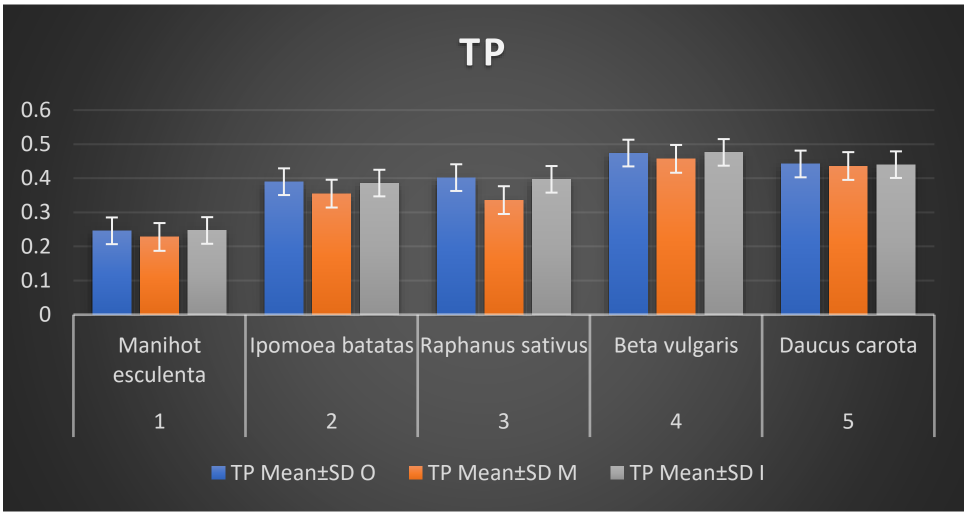

Figure 2: TP in Tubers

Overall, these findings provide a comprehensive anatomical–biochemical framework for evaluating oxalate and phenolic distribution in root vegetables. They support dietary recommendations for vulnerable populations and highlight the importance of region- and cultivar-specific profiling, considering environmental, nutritional, and processing influences[24]. Soluble oxalate and insoluble oxalate are in a dynamic equilibrium in a plant which on trigger of factors such as increase in external calcium composition, nitrate content and ammonia concentration reregulates the internal soluble and insoluble oxalate concentration to suit the external equilibrium[20]. He also deciphered that <20% soluble oxalate content should be contributed to total oxalate content for the plant to be fit for consumption by ruminants. Thus, the area occupied by crystals (Fig 3) and total oxalate load on comparison pointed to the amount of soluble oxalate in a plant. In soybean seeds it was reported that insoluble calcium oxalate crystals reduced from 24% to 18% during the process of seed development according to the change in physiological conditions[30]. This indicated the reduction in area occupied by crystals due to change in physiological conditions of the plant. The present study also reported change in area occupied by crystals at different layers of roots pointing to different physiological processes taking place in those areas.

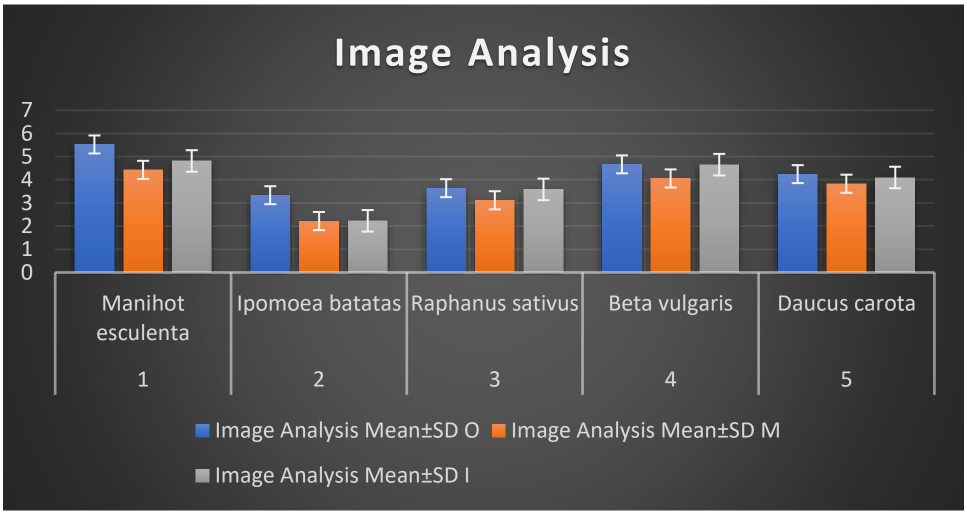

Figure 3: Image Analysis in Tubers

CONCLUSION

This study provides a comprehensive tissue-specific assessment of calcium oxalate crystal morphology, total oxalate content, phenolic distribution, and crystal density in selected edible root vegetables, addressing a major knowledge gap in the anatomical and biochemical profiling of roots. Crystal sand was the dominant crystal form in *Manihot esculenta*, *Beta vulgaris*, *Raphanus sativus*, and *Daucus carota*, while styloid crystals characterized *Ipomoea batatas*, reinforcing the taxonomic value of crystal morphology in roots.

Although total oxalate and total phenol contents did not differ significantly among the outer skin, middle cortex, and inner pith, pronounced interspecific variation was evident. *Daucus carota* consistently exhibited the highest total oxalate load across all tissues, exceeding recommended dietary limits, whereas *Beta vulgaris* remained within permissible levels, indicating greater dietary suitability. The elevated oxalate values observed compared with earlier reports highlight the strong influence of environmental and regional factors and underscore the need for localized oxalate databases.

In contrast, the area occupied by calcium oxalate crystals varied significantly across tissue layers, with the outer skin showing the highest crystal density. The inverse relationship between crystal area and total oxalate load, particularly in *Manihot esculenta* and *Beta vulgaris*, suggests a predominance of insoluble oxalate forms and reduced oxalate bioavailability. Overall, the integrated anatomical–biochemical approach adopted in this study provides valuable insights into nutritional safety, supports peel removal for vulnerable populations, and establishes a framework for future research aimed at improving the dietary quality of root vegetables.

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