Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Taro : Insights Into Nutrient Content and Anti-Nutritional Factors

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Cite this paper as: Renu Rajan, Justin R Nayagam, (2025) Taro : Insights Into Nutrient Content and Anti-Nutritional Factors. *Journal of Neonatal Surgery*, 14 (32s), 2354-2359.

ABSTRACT

Taro is a **nutritional powerhouse** rich in **fibre, vitamins A, C, E, and B-complex, and various minerals** such as potassium, magnesium, and iron. It is enjoyed as an international cuisine owing to its low-fat content, high fibre and protein content with moderate carbohydrate level, over many underground stem tubers and corms. Microscopic study in the present work reveals the presence of raphide bundles and druse calcium oxalate crystals distributed throughout the outer as well as middle cortex and inner pith of the edible corm. The total oxalate load of taro was deciphered to be more than twelve times its permissible nutritional level. Chemical analysis and spectrophotometric analyses confirmed that maximum TO was in the outer skin (6.825 mg/g sample) followed by inner pith (6.814 mg/g sample) and the least TO was found in the middle cortex. Image analysis results in *Colocasia esculenta* showed that there is difference in area occupied by crystals among the outer skin, middle cortex and inner pith. Maximum area of crystals was distributed among the inner pith or the core region of the underground stem vegetable succeeded by outer skin portion and the least distribution was observed to be the middle cortex where the area occupied by crystals were 626.910 $\mu m^2/cm^2$. The maximum phenol content was in the outer skin (0.377 mg GAE/g) of the underground stem vegetable, followed by inner pith (0.371 mg GAE/g) and least phenol content in the middle cortex (0.357 mg GAE/g). A protocol for the selection, improved varieties use of taro is therefore required with respect to its oxalate dominant area and intervention in the form of AI mediated predictions and genetic manipulation is promising in modifying the crop in future.

Keywords: *Taro, Colocasia esculenta, ergastic crystal, Total oxalate (TO), raphide bundle*

1. INTRODUCTION

Colocasia, commonly known as taro, is believed to have originated in Southeast Asia and the Indian subcontinent. It has been cultivated for thousands of years and is a staple food in many parts of Asia and Africa. In Africa, it is particularly popular in West and Central Africa, where it accounts for over 70% of global production (Quero- Garcia et al., 2010). The plant is a herbaceous annual found in tropical biome with characteristic sagittate leaves and belong to Araceae family. Leaves with long petiole arise from underground stem, lamina. Grown for the underground starchy tuber commonly called taro. Unisexual flowers are borne in a spadix inflorescence. Flowering and seed setting rare and hence propagated by underground stem (Nayagam, 2023). Taro accommodates ergastic crystals of calcium oxalate origin known to cause serious lip, mouth and throat swelling if consumed raw (Du Thanh et al., 2017). Cooking practices has an influence in the oxalate levels in taro (Adane et al, 2013). The corm is a reliable source of starch (70–80 g/100 g dry weight), fiber (0.80%), ash (1.2%), and fat (0.20%) but low in protein (1.5%) similar to many other tuber crops (Rashmi et al., 2018). The leaves are rich in nutritional and phytochemical compounds, however, they are underutilized.

Taro as a high nutrition value crop that can support in hunger eradication and SDGs of UN. The present study evaluates the nutritional antioxidant total phenol load of taro as well as the anti-nutritional oxalate load in its raw form, which has to be addressed as it can cause renal failure if consumed above permissible dietary levels.

2. MATERIALS AND METHOD

A plot of taro plant is readily available in the botanical garden of Union Christian College, Aluva, India (*10.12105 Latitude and 76.3308 Longitude)*. and the fresh sample for the present study was procured afresh. Samples from the authorised supermarkets and local markets were also used for cross checking the data obtained. The data thus obtained are compared.

Pizzolato Method: The working solution of the stain should be freshly prepared just prior to staining as light can deactivate the stain. Equal quantities of 30% hydrogen peroxide and 5% silver nitrate have been combined to make the working stain solution. Sections have been flooded with stain and slides were placed 6 inches away from a 60-watt light bulb for 30 min (Pizzolato, 1964). Sections were rinsed and the observation was made under the microscope to find black calcium oxalate crystals.

Estimation of Total Oxalate by Permanganometry: Estimation of oxalate load was done by permanganometric titration with standardized KMnO4 following the Association of Official Analytical Chemists procedures (AOAC, 2016)

Sample Preparation: To extract the total oxalate from plant samples, 1g homogenized fresh sample is added to 30ml of 0.5 NH2SO4 and make it boil in a water bath for the time of 15 min. The extract is now being filtered by utilizing the Whatman's No1 filter paper and an equal volume of deionized water is added to prepare the sample extract.

Oxalate ions are extracted from the plant parts by boiling them with dilute H2SO4 (0.5N). Then oxalate concentration was estimated volumetrically by titrating the extract with standard KMnO4 solution (0.05N). MnO4 - + + 5C2O4 +8H+ → Mn2+ + 10CO2 + 4 H2O. 1 ml of sample extract is added to 40 ml dil. H2SO4 in a conical flask and titrated against standard KMnO4 until a permanent pink coloration appears and stays for at least 15 seconds. The endpoint is noted and the amount of oxalate present in mg/sample is estimated stoichiometrically.

Estimation of Total Oxalate by Spectrophotometry: The kinetic Spectrophotometric method (Chamjangali et al., 2006) for observing the oxalate traces by using an activation effect was utilized for the estimation of total oxalate in samples. 3.4.2.1. Sample Preparation 2.5 g of sample cut into small pieces was pounded using a mortar and pestle and it was refluxed in a flask with 250ml distilled water for the time of 20 min. The cooled mixture has been filtered by using the Whatman No.1 filter paper and the filtrate has been diluted to 100ml. 2ml of this sample was used for analysis. In a 100ml volumetric flask, a stock ferrous iron solution of 1000µg/ml concentration has been synthesized by dissolving the Fe (NH4)2 (SO4)2 in the amount of 0.7021g. 6H2O in 0.10 mol/l H2SO4. A stock iodide solution of concentration 0.120 mol/l has been synthesized by dissolving potassium iodide in the amount of 2.0021g in a 100ml volumetric flask making up to the volume with distilled water.

A stock bromates solution of concentration 0.100mol/l has been synthesized by dissolving sodium bromate in the amount of 1.5090g in distilled water in a 100ml volumetric flask. An acetate buffer of pH 5 has been synthesized by the mixing of appropriate quantities of acetic acid (0.20mol/l) and sodium acetate (0.20mol/l) until maintaining the pH. Shimadzu UV-1800 spectrophotometer (Fig 3.4.2) with quartz cuvettes was used for the spectrophotometric analysis of samples. The reaction mixture was made by adding 2ml samples,2ml acetate buffer,1 ml ferrous iron solution,1 ml potassium iodide, and 1ml sodium bromate solution in a 10ml standard flask and making up with distilled water to 10 ml. This was poured into a quartz cuvette as a sample and the blank was kept with plain distilled water. The increase in absorbance at 352nm was noted from 0.5-4 mins from the initiation of reaction (ΔAs) which is the sample value. Now the process is repeated in the absence of a sample to get the blank value (ΔAb). Oxalate concentration in the sample=ΔAsΔAb.

Estimation OF Total Phenol Content by Folin Ciocalteu Mehod: Total phenol estimation of all samples was performed following the modified Folin- Ciocalteu method (Ainsworth, 2007). The calibration curve was plotted using Gallic acid as standard.

Plant parts were crushed using mortar and pestle by weighing and dissolving in distilled water to 100µg/ml concentration. Folin -Ciocalteu reagent was diluted to a 1:10 ratio with de-ionized water. After combining 0.5 ml of the plant extract with 2 ml of the diluted Folin-Ciocalteu reagent, we then neutralized the mixture using 4 ml of a solution of sodium carbonate at a concentration of 7.5%. The reaction mixture has been intermittently shaken and incubated for the time of 30mins at room temperature for the development of a blue coloration. The absorbance of the blue colour was measured using Schimadzu-UV-1800 Spectrophotometer. Blanks were prepared by adding all the reagents except the sample but distilled water as a substitute. The linear equation of a standard curve that was plotted by different amounts of gallic acid was used in this approach to calculate the total phenol content of a sample. The linear equation was derived from the data. The amount of total phenol is measured in milligrams/millilitres of gallic acid. The reduction of Folin Ciocalteu reagent with phenolic compounds results in the formation of molybdenum-tungstate blue which can be measured at 565 nm.

3. RESULTS

Crystal Characterisation in *Colocasia esculenta*

The calcium oxalate crystals found in *Colocasia esculenta (L.) Schott* were crystal raphides and druses (Fig.3). The crystal raphides was found in bundles in all parts of the underground stem vegetable such as outer skin, middle cortex and inner pith. The crystals were transparent, crystalline needle shaped with pointed tips on both sides dispersed in idioblast cells in the tissue. Druses with no idioblasts were also visible. Pizzolato staining (Fig. 4) stained calcium oxalate crystals to black colour. Crystals were soluble in 2% HCl with no gas emission after showing no solubility in 5% acetic acid which confirmed calcium oxalate content of the raphide and druse crystals.

Estimation of Total Oxalate by Permanganometry in *Colocasia esculenta*

Permanganometric estimation (Table 1) of Total oxalate in *Colocasia esculenta (L.) Schott* showed that there is a difference in distribution in the TO levels at the outer (O) skin, middle (M) cortex and inner (I) pith of the underground stem vegetable. Maximum TO was in the outer (O) skin (6.825 mg/g sample) followed by (I) inner pith (6.814 mg/g sample) and the least TO was found in the middle (M) cortex.

Table 1. Total oxalate in *Colocasia esculenta* by permanganometry

Samples	Samples	Minimum	Maximum	Mean±SD	CV%
Colocasia esculenta	**O**	6.823	6.827	6.825±0.002[a]	0.03
	M	6.782	6.802	6.794±0.009[b]	0.13
	I	6.798	6.824	6.814±0.010[a]	0.15

One way ANOVA analysis showed that the maximum variation among the samples was in outer skin (O) (6.823-6.827 mg/g) and inner pith (I) (6.798- 6.824 mg/g) denoted by superscript 'a'. Middle (M) cortex (6.782- 6.802 mg/g) showed least variation denoted by superscript 'b'

Estimation of Total Oxalate by Spectrophotometry in *Colocasia esculenta*

Table 2. Total oxalate in *Colocasia esculenta* by spectrophotometry

Samples	Samples	Minimum	Maximum	Mean±SD	CV%
Colocasia esculenta	**O**	6.825	6.845	6.835±0.007[a]	0.10
	M	6.782	6.900	6.813±0.050[a]	0.73
	I	6.714	6.914	6.795±0.083[a]	1.22

Spectrophotometric Total Oxalate estimation in *Colocasia esculenta (L.) Schott* showed (Table 2) that highest oxalate load was in (O) outer skin (6.835 mg/g sample) followed by a decrease in the (M) middle cortex (mg/g sample) and the minimum oxalate load was found in the inner (I) pith (6.795 mg/g sample). One way ANOVA results revealed that variation among samples were in the same range depicted by superscript 'a'. Variance present between the outer, middle and pith region was similar in *Colocasia esculenta* (L.) Schott.

Estimation of Total Phenol Content in *Colocasia esculenta*

In *Colocasia esculenta (L.) Schott* the total phenol (Table 3.) estimation results showed that maximum phenol content was in the (O) outer skin (0.377 mg GAE/g) of the underground stem vegetable, followed by (I) inner pith (0.371 mg GAE/g) and least phenol content in the (M) middle cortex (0.357 mg GAE/g). One way ANOVA results showed that there is variation among samples studied and that maximum variation was among (O) outer skin (0.375- 0.379 mg GAE/g) denoted by superscript 'a' followed by lesser variation in (I) inner pith (0.369- 0.372 mg GAE/g) denoted by superscript 'b'. Middle cortex (M) showed least variation (0.356- 0.372 mgGAE/g) compared to others denoted by superscript 'c'.

Table 3. Total phenol in *Colocasia esculenta* by Folin- Ciocalteu method

Samples	Samples	Minimum	Maximum	Mean±SD	CV%
Colocasia esculenta	**O**	0.375	0.379	0.377±0.002[a]	0.53
	M	0.356	0.358	0.357±0.001[c]	0.28
	I	0.369	0.372	0.371±0.002[b]	0.54

Image Analysis in *Colocasia esculenta*

Table 4. Image analysis in *Colocasia esculenta*

Samples	Samples	Minimum	Maximum	Mean±SD	CV%
Colocasia esculenta (L.) Schott	**O**	630.215	635.298	632.308±1.94[a]	0.31
	M	623.245	630.015	626.910±2.72[b]	0.43
	I	630.258	637.334	633.241±3.13[a]	0.49

Image analysis results in *Colocasia esculenta (L.) Schott* showed that (Table 4.) there is difference in area occupied by crystals among the outer skin (O), middle cortex (M) and inner pith (I). Maximum area of crystals was distributed among the inner pith (I) or the core region of the underground stem vegetable succeeded by outer skin (O) portion and the least distribution was observed to be the middle cortex (M) where the area occupied by crystals were 626.910 μm^2. One way ANOVA results showed that the variation among samples were highest in outer (O) skin (630.215- 635.298 μm^2) and inner pith (I) (630.258- 637.334 μm^2) denoted by superscript 'a'. The least variation among samples were observed in (623.245-630.015 μm^2) the middle cortex (M).

4. DISCUSSION

Catherwood et al. (2007) reported total oxalate in Akame taro as 171.4 mg/100g, which is lower than observed TO content in Colocasia esculenta in present study. It may be due to difference in cultivar or difference in environmental conditions grown. In Colocasia corms, dynamic nature of calcium oxalate crystals was reported by Sunell and Haley (1979). They could explain highest density of crystals in the growing stage to maturity, followed by decline after senescence. The level of oxalates varies according to corm part and cultivar. Water-soluble oxalates accumulate mainly in the central and lower parts, while insoluble oxalates are concentrated in the marginal part of the corm (Kristl *et al*., 2021). Their content can be almost halved by removing a 1 cm thick marginal layer. Catherwood *et al.* reported (2007) that cooking practices such as boiling has an advantage over baking in Japanese taro in reducing total oxalate. Variation in taro oxalate content is consistent and significantly correlated with the photosynthetic rate, carbohydrate metabolism and protein synthesis (Gouveia *et al*., 2018).

Physiological changes in plants such as proliferation of pseudo stem structures was found associated with increased size of druse crystals present in *Curcuma longa* (Abraham et al., 2022) during maturation of the plant. Previous studies on various layers of underground stems were not available for comparison. Comparison of total oxalate content in inner pith (I) of taro showed that permanganometric and spectroscopic values were comparable (Fig.1). Inner pith TO in *Colocasia esculenta* (681.4mg/100g) showed very high oxalate load than permissible TO level.

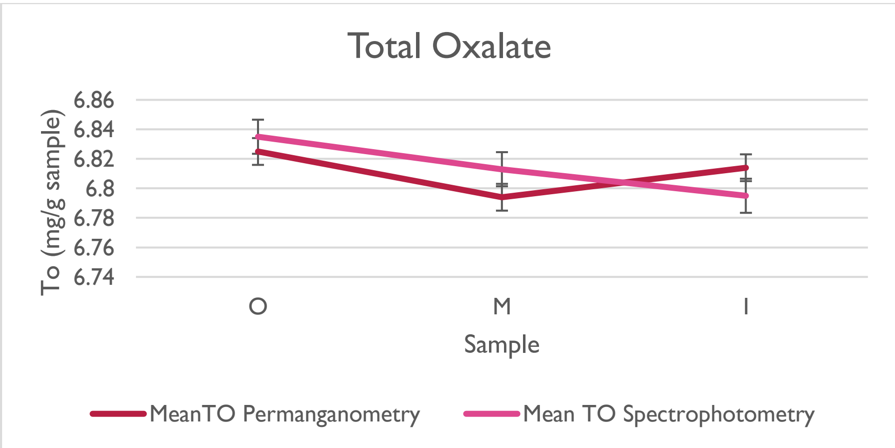

Fig. 1: Total Oxalate estimation in Taro

Comparison of total phenol content (Fig. 2) in the outer skin of underground stem showed that *Colocasia esculenta* (37.7 mg GAE/100g) showed TP content in the range of 30- 40 mg GAE/100g.

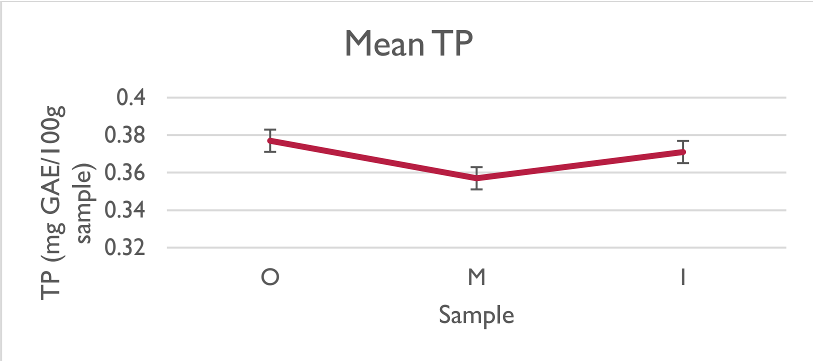

Fig.2: Mean Total Phenol in Taro

Total phenol content in vegetables vary depending on the climatic factors, stage of ripeness, geographical distribution and storage practices (Goncalves et al., 2004) (Wang, 2006). Antioxidant activity in root tubers of India studied by Sreeramulu and Reghunath (2010) also pointed to the difference in total phenol content of vegetables grown in different regions and climatically varied regions. Comparison of area occupied by calcium oxalate crystals in the outer skin (O) of underground stem vegetables showed that very high area occupancy of crystals was observed in *Colocasia esculenta* (632.308 $\mu m^2/cm^2$) within a range of 600- 800 $\mu m^2/cm^2$. Image analysis data showed a dip in the crystal occupied area of middle cortex in taro than outer skin and inner pith region. This could be a noteworthy information for renal patients in choosing a low TO region of taro in low oxalate diet preferences.

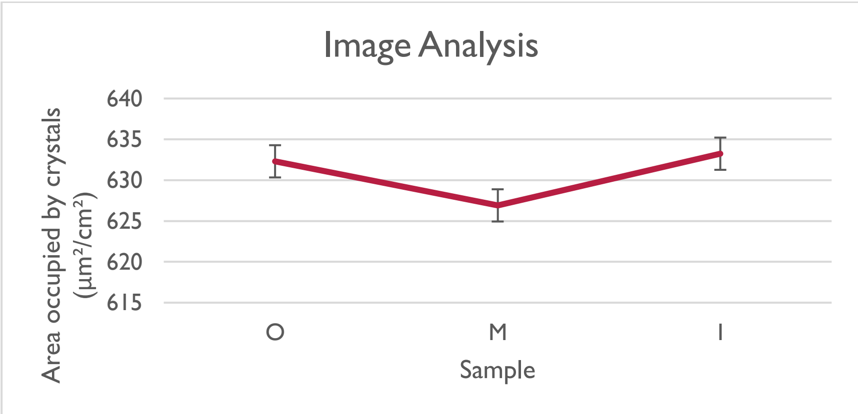

Fig.3: Image Analysis in Taro

5. CONCLUSION

The present study concluded presence of raphide bundles and druse calcium oxalate crystals distributed throughout the outer as well as middle cortex and inner pith of the edible corm. The total oxalate load of taro was deciphered to be more than twelve times its permissible nutritional level (50mg/100g sample per day). Permanganometric and spectrophotometric analyses confirmed that maximum TO was in the outer skin (6.825 mg/g sample) followed by inner pith (6.814 mg/g sample) and the least TO was found in the middle cortex. Image analysis results in *Colocasia esculenta* showed that there is difference in area occupied by crystals among the outer skin, middle cortex and inner pith. Maximum area of crystals was distributed among the inner pith or the core region of the underground stem vegetable succeeded by outer skin portion and the least distribution was observed to be the middle cortex where the area occupied by crystals were 626.910 $\mu m^2/cm^2$. The maximum phenol content was in the outer skin (0.377 mg GAE/g) of the underground stem vegetable, followed by inner pith (0.371 mg GAE/g) and least phenol content in the middle cortex (0.357 mg GAE/g). The total oxalate load is way more than the permissible amount of consumable oxalate per day in taro, hence the portion serving, frequency of consumption and part of consumption need to be addressed as it can impair the renal health of people with renal ailments.

Acknowledgements

The authors express their gratitude to the research department of Botany, Union Christian College, Aluva for their support and encouragement during the study.

Competing Interests

Authors have declared that no competing interests exist.

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