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ESTIMATION OF NUTRITIONAL PARAMETERS OF INDOTRISTICHUM RAMOSISSIMA OF TRISTICHOIDEAE (PODOSTEMACEAE)

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Abstract

Indotristichum ramosissima (Podostemaceae) is an aquatic rheophytic plant adapted to fast-flowing freshwater environments. Despite its ecological significance, there is limited research on its nutritional composition. This study aims to comprehensively analyze the nutritional profile of *I. ramosissima* by estimating key parameters, including carbohydrates, proteins, fats, crude fiber, tocopherol, vitamin C, ash content and elemental composition. Standard biochemical assays were employed for quantitative estimations to ensure accuracy and reliability. The findings reveal the plant's notable nutritional potential, suggesting its possible applications in food and medicinal fields. The presence of essential nutrients and bioactive compounds like tocopherol and vitamin C highlights its value as a potential functional food source. Furthermore, the elemental composition provide insights into its phytochemical properties, warranting further pharmacological investigations. With increasing global interest in underutilized aquatic plants for their nutritional and medicinal benefits, this study contributes to the growing body of knowledge on Podostemaceae. By shedding light on the biochemical attributes of *I. ramosissima*, this research provides a foundation for future studies exploring its therapeutic potential, conservation strategies, and sustainable utilization in nutraceutical and pharmaceutical applications.

Keywords: Aquatic plants, *Indotristichum ramosissima*, Nutritional estimation, Phytochemistry, Podostemaceae.

Introduction

Aquatic angiosperms belonging to the family Podostemaceae are uniquely adapted to extreme freshwater environments such as rapidly flowing rivers and waterfalls. These plants exhibit specialized morphological and physiological adaptations that allow them to attach firmly to submerged rocks and withstand strong water currents. One such species, *Indotristichum ramosissima*, is a rheophytic aquatic plant restricted to specific riverine ecosystems. Despite its ecological importance and specialized habitat, this species remains relatively understudied, particularly with respect to its biochemical composition and potential uses.

Aquatic plants are increasingly recognized as valuable sources of nutrients and biologically active compounds with potential applications in food, nutraceutical, and medicinal fields. Previous research on various aquatic taxa has demonstrated the presence of essential macronutrients, micronutrients, and secondary metabolites that may possess antioxidant, antimicrobial, and anti-inflammatory properties. However, limited information is available regarding the nutritional and elemental composition of members of the Podostemaceae family, including *Indotristichum ramosissima*. Therefore, the present study aims to evaluate the nutritional profile of this species by estimating carbohydrate, protein, fat, crude fiber, tocopherol, vitamin C, ash content, and extractive values through standard biochemical assays, along with an analysis of its elemental composition. This investigation seeks to provide baseline data on the nutritional and bioactive potential of the plant and to support future phytochemical and pharmacological studies.

Materials and Methods

Collection and Preparation of Plant material

Fresh specimens of *Indotristichum ramosissima* were collected from riverine habitats in Kallar river (Lat 9.240924^0 & Long 76.965226^0) a tributary of Pampa, Pathanamthitta district, Kerala. The collected specimen was identified with Flora of the Presidency of Madras, Gamble (1997); verified with Herbarium of Department of Botany, Calicut University and confirmed with the help of experts and by Molecular studies. The collected samples were washed with distilled water to remove debris,

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dried at room temperature for 7 days.

Estimation of Nutritional parameters

Analysis on the presence of different nutritional parameters such as carbohydrates, proteins, fat, crude fibre, tocopherol and vitamin C were analysed according to the standard procedures, as described below.

Estimation of Carbohydrate by Phenol – Sulphuric acid method (Sadasivam, 1996)

Preparation of sample: Dry sample were crushed in pestle and mortar, from this 10 mg weighed and taken in boiling tube. Add 0.5 ml of 2.5N HCl and boiling tubes were kept in water bath for 3hrs and then cooled to room temperature. After cooling it was neutralized by adding sodium carbonate until effervescence ceases. Then final volume was made up to 10 ml by adding distilled water and centrifuged. Supernatant was used as sample in further process.

Procedure: A 0.2,0.4,0.6,0.8 and1ml of working standard (with 1mg/ml glucose stock) of glucose was taken in boiling tubes and the final volumes of each tube was made up to 1ml by adding distilled water.1ml of 5% Phenol and 5ml of 96% sulphuric acid was added one by one in each tube and shook well so that the Phenol and Sulphuric acid get mixed thoroughly with working standard. After 10 minutes all the tubes were placed in water bath at 25-30°C for 15 minutes. Blank was set with 1ml of distilled water and O.D. of each tube was taken at 490nm with the help of spectrophotometer. Appropriate sample volumes were taken for carbohydrate analysis and procedures were same as for standard.

Estimation of Protein by Lowry's method (Lowry *et al.*, 1951)

Preparation of sample: Homogenize 100 mg sample in 5 ml of freshly prepared phosphate buffer saline of pH7.4. Centrifuge at 10000 rpm for 10 min and collect final supernatants in respective tubes.

Procedure: Samples, standard and blank were pipetted in respective tubes. To this add 1mL alkaline copper reagent. Then incubate for 10min at room temperature. Then add 100µL Folin's reagent. Incubate for 30min at room temperature. After incubation read OD at 660nm with the help of spectrophotometer.

Estimation of Fat by Acid Hydrolysis (AOAC, 1980)

Procedure: Grind the dried sample through a 1mm sieve. Weigh 1-gram ground sample into a 50 ml screw-top test tube. Wet sample with 1 ml of ethanol, saturating it. Add 5 ml HCl. Place it in a preheated water bath (75.5^0C) for 40 minutes. Shake occasionally. Remove and allow cooling to room temp. Add 5 ml ethanol and mix. Then add 12 ml anhydrous ether (orbital shake for 1 minute) and add 12 ml petroleum ether (orbital shake for 1 minute). Let ether and residue separate. Pull off top layer into a dried and tared 150 ml beaker via a Pasteur pipette, pouring through a filter paper in a long stem funnel. Evaporate ether and any water contained in beaker. Place beakers in a 135^0C oven for 10 minutes. Transfer to desiccator and allow cooling to room temperature. Weigh beaker plus fat to +/-0.01g.

Calculations:

Percentage of Fat = ((Weight of beaker and fat – tared beaker weight) / Sample weight)) x100

Estimation of Crude Fibre (Maynard, 1970)

Procedure: Extract 2g of ground material with ether or petroleum ether to remove fat. After extraction with ether, boil 2g of dried material with 200mL of sulphuric acid for 30 min with bumping chips. Filter through muslin and wash with boiling water until washing are no longer acidic. Boil with 200 mL of sodium hydroxide solution for 30min. Remove the residue and transfer to ashing dish (pre-weighed dish W1). Dry the residue for 2h at 130 ±2°C. Cool the dish in a desiccator and weigh (W2). Ignite for 30min at 600 ±15°C. Cool in a desiccator and reweigh (W3).

Calculation:

Percentage of crude fiber in ground sample =
Loss in weight on ignition (W2 - W1) – (W3 - W1) ÷ Weight of the sample × 100

Estimation of Tocopherol (Vitamin E) by Ranganna (1997)

Homogenize samples (0.5g) in 10 ml of 0.1N sulphuric acid and make up the volume up to 50 ml by adding 0.1N sulphuric acid slowly, without shaking. Allow the contents to stand

overnight. Shake the flask vigorously on the next day and filter through Whatman No.1 filter paper. Pipette out 1.5ml of sample, standard and water into three centrifuge tubes namely test, standard and blank respectively. Add 1.5ml each of ethanol and xylene and mix well. After centrifugation, transfer the xylene layer into another tube, taking care not to include any ethanol or protein. Add 1ml of 2, 2′-dipyridyl reagent (0.12% in iso propanol) to 1 ml of xylene layer and mix the solution. This reaction mixture was taken in the spectrophotometric cuvettes. Read the test and the standard against the blank at 460nm. Then add 0.33ml of ferric chloride solution (0.12 % in ethanol) and mix well. After 15 minutes, read the test and the standard against the blank at 520nm.

The levels of tocopherol were calculated using the formula:

$$\text{Tocopherol } (\mu g) = \left[\frac{A520\text{-}A460}{StdA520}\right] \times 0.29 \times 15 \times \left[\frac{\text{Total volume of homogenate}}{\text{Volume used} \times \text{weight of the tissue}}\right]$$

Estimation of Vitamin C (Suntornsuk *et al.*, 2002)

Procedure: Add 25.00 ml of vitamin C standard solution to a 125 ml Erlenmeyer flask. Add 10 drops of 1% starch solution. Rinse your burette with a small volume of the iodine solution and then fill it. Record the initial volume. Titrate the solution until the endpoint is reached. This will be when you see the first sign of blue color that persists after 20 seconds of swirling the solution. Record the final volume of iodine solution. The volume that was required is the starting volume minus the final volume. Repeat the titration at least twice more. The results should agree within 0.1 ml.

Estimation of Ash content and Elemental analysis

Estimation of Ash content (Keshun Liu, 2019)

The procedure for measuring ash content consisted of the following steps:

pre-conditioning porcelain crucible in a muffle furnace under a vent hood at an ashing temperature of 600 °C for at least 30 min, to remove any combustible contaminants; 2) removing crucible from the furnace and cooling to room temperature in a desiccator (about 1 h); 3) weighing crucible to the nearest 0.1 mg, using gloves, tweezers, or tongs to prevent adding weight from hand moisture and contaminants; 4) weighing 1–4 g of powdery sample (enough to produce 20 mg or more of ash) into the tared crucibles 5) placing the crucible with sample in the muffle furnace that had been set and pre-heated to a temperature of 600 °C under a vent hood; 6) pre-igniting sample by spontaneous auto combustion 7) ashing the samples in the furnace overnight (about 16 h); 8) removing the crucible with ashed sample from the muffle furnace for cooling to room temperature in a desiccator; 8) weighing crucible with ash; and 9) calculating the total ash content as % sample mass.

Elemental analysis using AAS (Valkovic, V.V., 1975)

The plant sample was first cleaned dried and then powdered using a electric blender. Sample in powder form were used for Atomic Absorption Spectrophotometer (AAS). Plant material (0.25 g) were taken in 50 ml flask and add 6.5 ml of mixed acid solution that is, Nitric acid (HNO3), Sulfuric acid (H2SO4) and Perchloric acid (HClO4) (5:1:0.5) The sample boiled in acid solution in fume hood on hot plate till the digestion has been completed which was indicated by white fumes coming out from the flask.

Thereafter, few drops of distilled water were added and allowed to cool. Then these digested samples were transferred in 50 ml volumetric flasks and the volume was made up to 50 ml by adding distilled water in them. Then filter the extract with filter paper (Whatmann No. 42) and filtrate were collected in labeled plastic bottles. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer Shimadzu AA-670 with suitable hollow cathode lamps. The percentages of different elements in these samples were determined by the corresponding standard calibration curves.

Results and Discussion

Nutritional Composition and Ash content of *Indotristichum ramosissima*

The study revealed that *I. ramosissima* contains significant amounts of carbohydrates (0.146 ± 0.055%), proteins (5.736 ± 2.697%), and fats (3.01 ± 0.196%). The crude fiber content was recorded at 0.953 ± 0.055%, indicating its potential as a dietary fiber source. The plant exhibited considerable levels of tocopherol (36.126 ± 1.088 mg/g) and vitamin C (1.312 ± 0.156 mg/g), which contribute to its antioxidant potential. The total ash content was 15.10± 1.65%, reflecting its mineral-rich nature. The nutritional analysis of the studied sample of *Indotristichum ramosissima* reveals a diverse composition of essential macronutrients and micronutrients, which may contribute significantly to its dietary and medicinal value.

The carbohydrate content was found to be 0.146 ± 0.055 g per gram of sample, indicating a relatively low presence of carbohydrates. This suggests that the sample may not be a primary energy source but may complement other nutrient-rich foods in a balanced diet (Smith *et al*., 2020). Protein content was recorded at 5.736 ± 2.697 mg per gram, highlighting its potential contribution to amino acid requirements, which are crucial for cellular function and growth (Johnson & Patel, 2018). While this protein content is moderate, it may still serve as an additional source of dietary protein when consumed regularly.

One of the most remarkable findings was the high tocopherol content, recorded at 36.126 ± 1.088 mg per gram of sample. Tocopherols, particularly vitamin E, play a significant role as antioxidants, protecting cells from oxidative stress and contributing to skin health, immune function, and neurological well-being (Brown *et al.*, 2019). The substantial presence of tocopherol in *I. ramosissima* suggests potential antioxidant properties that could be explored further for therapeutic applications.

The vitamin C content was found to be 1.312 ± 0.156 mg per gram, indicating its contribution to immune support, collagen synthesis, and free radical scavenging (Lee & Kim, 2021). Although lower than some vitamin C-rich fruits and vegetables, this presence still signifies its importance in enhancing antioxidant capacity and promoting overall health.

Crude fiber, an essential dietary component, was estimated at 0.953 ± 0.055%. Dietary fibre plays a key role in digestive health, promoting gut motility, reducing cholesterol levels, and aiding in glycemic control (Anderson *et al.*, 2017). Though the fiber content in the sample is modest, its inclusion in the diet could provide digestive benefits.

Fat content was recorded at 3.01 ± 0.196%, which is a notable proportion. This could contribute to the sample's caloric value and essential fatty acid supply, which are vital for cellular membrane integrity and metabolic functions (Garcia & Lopez, 2022). The nature of these fats, whether saturated or unsaturated, requires further analysis to determine their impact on cardiovascular health.

The ash content, which represents the total mineral content, was found to be 15.10 ± 1.65%. This suggests a significant presence of minerals that could include essential elements such as calcium, magnesium, potassium, and trace elements necessary for enzymatic activities, bone health, and metabolic functions (Miller *et al.*, 2020). The high ash value implies that *I. ramosissima* might be a good source of dietary minerals, contributing to overall nutritional adequacy. The results are shown in Table.1.

Table 1. Nutritional composition and Ash content

Nutritional analysis	Quantity in 1gm sample
Carbohydrate (gm)	0.146 ± 0.055
Protein (mg)	5.736 ± 2.697
Tocopherol (mg)	36.126 ± 1.088
Vitamin C (mg)	1.312 ± 0.156
Crude fibre (%)	0.953 ± 0.055
Fat (%)	3.01 ± 0.196
Ash content (%)	15.10± 1.65

Elemental analysis using AAS

The elemental analysis of *Indotristichum ramosissima* was conducted using Atomic Absorption Spectroscopy (AAS)

to determine the concentration of essential and trace minerals. The results revealed significant variations in the mineral composition, with notable levels of iron, potassium, zinc, and manganese, which are essential for various physiological and biochemical functions in plants and humans.

Iron (Fe) was found at a mean concentration of 6.638 ± 0.59 ppm. Iron is a crucial micronutrient necessary for oxygen transport and enzymatic processes (Kabata-Pendias & Pendias, 2011). The relatively high Fe content in *I. ramosissima* suggests its potential role in iron supplementation, particularly in iron-deficient diets. Potassium (K), an essential macronutrient, was the highest among all analyzed elements, with a mean concentration of 7.342 ± 0.16 ppm. Potassium plays a vital role in plant osmoregulation and enzyme activation, as well as in maintaining electrolyte balance in humans (Marschner, 2012).

Zinc (Zn) and manganese (Mn) were also present in notable concentrations, with mean values of 3.820 ± 0.10 ppm and 3.893 ± 0.11 ppm, respectively. Zinc is an essential trace element involved in enzymatic functions and immune responses (Alloway, 2008), while manganese is crucial for photosynthesis, antioxidant activity, and neurological health (Broadley *et al.*, 2012). The observed levels of these elements indicate that *I. ramosissima* could be a valuable source of Zn and Mn for dietary and medicinal applications. The presence of sodium (Na) at a mean of 0.805 ± 0.02 ppm suggests its role in ionic balance and plant metabolism. Magnesium (Mg), recorded at 2.648 ± 0.05 ppm, is a critical component of chlorophyll and plays a role in enzyme activation and ATP metabolism (White & Broadley, 2009).

The study also detected trace amounts of lead (Pb) (0.064 ± 0.02 ppm) and cadmium (Cd) (0.013 ± 0.01 ppm), which are heavy metals with potential toxic effects when accumulated beyond permissible limits (Järup, 2003). However, the levels detected in *I. ramosissima* are relatively low and fall within safe consumption limits as per WHO/FAO guidelines (WHO, 2011). Selenium (Se) was found at a mean concentration of 1.680 ± 0.20 ppm, signifying its potential antioxidative and immunomodulatory properties (Rayman, 2012).

The negligible presence of chromium (Cr) at 0.002 ± 0.00 ppm indicates that *I. ramosissima* does not accumulate high levels of this element, which aligns with previous findings on chromium uptake in aquatic plants (Sinha & Pandey, 2003). The low concentration of copper (Cu) (0.050 ± 0.02 ppm) also suggests its limited accumulation, though Cu is essential for plant enzymatic activities (Marschner, 2012). The results are shown in Table.2.

Table 2. Elemental analysis using AAS

Mineral	Mean value in ppm
Copper	0.050 ± 0.02
Zinc	3.820 ± 0.10
Manganese	3.893 ± 0.11
Iron	6.638 ± 0.59
Lead	0.064 ± 0.02
Sodium	0.805 ± 0.02
Cadmium	0.013 ± 0.01
Selenium	1.680 ± 0.20
Potassium	7.342 ± 0.16
Chromium	0.002 ± 0.00
Magnesium	2.648 ± 0.05

Conclusion

This study highlights the significant nutritional potential of *Indotristichum ramosissima*, particularly its high tocopherol content, moderate protein and fat levels, and notable ash values indicating a rich mineral presence. These findings suggest its potential application in functional foods, dietary supplements, and pharmaceuticals. While its lower carbohydrate and fiber content make it less suitable as a primary energy source, it remains a promising antioxidant and protein supplement. Tocopherol, a key component in *I. ramosissima*, serves as a potent antioxidant that protects against oxidative stress and chronic diseases. As a natural source of vitamin E, it may offer benefits for skin health,

cardiovascular function, and immune support. The high mineral composition, including potassium, iron, zinc, manganese, and selenium, further enhances its nutritional significance. These minerals play essential roles in various physiological processes, including immune function, metabolism, and cardiovascular health. The detection of trace heavy metals, such as lead and cadmium, emphasizes the need for further toxicological evaluations to ensure safety for human consumption. However, the minimal levels of chromium and copper support its potential as a safe dietary component. Overall, *I. ramosissima* exhibits considerable nutritional and medicinal value. Further research on nutrient bioavailability, therapeutic effects, and sustainable cultivation is warranted to explore its full potential. With its rich antioxidant and mineral profile, this plant could contribute to the growing demand for natural health supplements and functional foods.

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References

Alloway, B.J (2008). Zinc in soils and crop nutrition, International Zinc Association, 2nd Edition, IZA and IFA, Brussels, Belgium and Paris, France.

Anderson, J.W, Baird, P, Davis, R.H. and Ferreri, S. (2017). Health benefits of dietary fiber, *Nutrition Reviews,* 75(3):20-30.

AOAC. (1980). Official Methods of Analyses.p.132.

Bhatt, S, Chandra, K. and Joshi, B. (2019). Nutritional analysis of lesser-known aquatic plants. *Journal of Aquatic Plant Studies,* 45(2):112-125.

Broadley, M, Brown, P, Cakmak, I, Rengel, Z. and Zhao, F. (2012). Function of nutrients: Micronutrients. In P. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants*. Academic Press. 191-248.

Brown, K, Thompson, M. and Green, P. (2019). Role of tocopherols in human health: A review. *Journal of Nutritional Sciences,* 8(4):45-60.

Garcia, R. and Lopez, J. (2022). Fat composition and its effects on human metabolism. *Food Chemistry,* 250:112-120.

Gamble, J.S. (1997). Flora of the Presidency of Madras Vol. II. Bishen Singh Mahendrapal Singh, New Connaught Place, Dehra Dun, India.

Gopalan, C, Rama Sastri, B.V. and Balasubramanian, S.C. (2004). *Nutritive Value of Indian Foods*. National Institute of Nutrition, ICMR.

Harborne, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman & Hall. 49-188.

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin, 68*(1):167-182.

Johnson, L. and Patel, R. (2018). The significance of dietary proteins in metabolic processes. *Journal of Food Science and Technology, 55*(2):78-90.

Kabata-Pendias, A. and Pendias, H. (2011). Trace elements in soils and plants. CRC press.

Keshun Liu. (2019). Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass, *Algal Research,* 40:101486.

Koi, S, Ikeda, H, Rutishauser, R. and Kato, M, (2015). Historical biogeography of river-weeds (Podostemaceae), *Aquatic Bot*any, 127:62–69.

Lee, S. and Kim, J. (2021). Vitamin C: Its role in health and disease prevention. *International Journal of Food Sciences and Nutrition,* 72(1):15-28.

Lowry, O.H, Rosebrough, N.J, Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193(1):265-275.

Marschner, P. (2012). *Marschner's Mineral Nutrition of Higher Plants*. Academic Press.

Maynard, A.J. (1970) Methods in Food Analysis Academic Press New York, 24:176.

Miller, D, Jackson, T. and Cooper, H. (2020). Essential minerals in human nutrition: Sources and bioavailability. *Annual Review of Nutrition,* 40(1):99-120.

Philbrick, C.T. and Novello, K.A. (1995). New World Podostemaceae: Ecological and evolutionary enigmas, *Briittonia*, 47(2):210-222.

Ranganna S. (1997). Handbook of Analysis and Quality Control for Fruit and Vegetable Products, 2nd Ed. Tata McGraw Hill publishing Co. New Delhi, India.

Rayman, M.P. (2012). Selenium and human health. *The Lancet,* 379(9822):1256-1268.

Sadasivam S. (1996). Biochemical methods. New age international.

Sinha, S. and Pandey, K. (2003). Accumulation of metals in *Aquatic macrophytes* and evaluation of bioindicators. *Environmental Toxicology,* 18(2):123-128.

Smith, R, Wang, Y. and Zhou, L. (2020). Carbohydrate metabolism and dietary intake. *Journal of Nutritional Biochemistry,* 34(5):120-135.

Suntornsuk, L, Gritsanapun, W, Nilkamhank, S. and Paochom, A. (2002). Quantitation of vitamin C content in herbal juice using direct titration. *Journal of pharmaceutical and biomedical analysis,* 28(5):849-55.

Valkovic, V.V. (1975). Trace Element Analysis. London: *Taylor and Francis*, 5-83.

White, P.J. and Broadley, M.R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets. *New Phytologist,* 182(1):49-84.

World Health Organization (WHO). (2011). *Guidelines for drinking-water quality*. WHO Press.

Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts. *Biochemical Journal,* 57 (3):508-514.