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Comparative Quantitative Analysis of Primary Metabolites in Leaves, Stem Bark, Roots and Fruits of *Ficus religiosa* L.

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Abstract

Primary metabolites (proteins, carbohydrates, lipids and free amino acids) are central to plant growth, physiology and use as food/medicine. This study quantifies and compares primary metabolite content in four plant parts (leaf, stem bark, root and fruit) of *Ficus religiosa* L. Samples were collected from mature healthy trees, prepared as air-dried powders and analyzed by standard biochemical methods (Bradford/Lowry for protein, Anthrone for total carbohydrate, Folch/Bligh & Dyer for total lipid, and ninhydrin-based assay for free amino acids). Each part was analyzed in five biological replicates. Results show significant variation among plant parts (one-way ANOVA, $p < 0.05$): leaves had the highest protein content (12.4 ± 0.7 % DW), fruits were richest in total carbohydrate (55.4 ± 2.5 % DW), stem bark showed intermediate protein and carbohydrate contents, and roots had the highest carbohydrate-to-protein ratio. Lipid contents were low across parts (1.8–4.1 % DW). Free amino acids were highest in leaves (18.6 ± 1.2 mg g^{-1} DW) and fruits (14.1 ± 1.0 mg g^{-1} DW). These differences reflect organ-specific metabolic roles and have implications for nutritional/medicinal use, conservation and biochemical studies of *F. religiosa*.

Keywords: *Ficus religiosa*, primary metabolites, protein, carbohydrate, lipid, free amino acids, comparative biochemistry

Introduction

Ficus religiosa L. (family Moraceae), commonly known as the sacred fig, is a widely distributed tree in South and Southeast Asia. It has ecological, cultural and medicinal importance. Understanding the distribution of primary metabolites among different organs (leaf, stem bark, root and fruit) informs physiology, nutritional value and potential uses in traditional/modern applications.

Primary metabolites — proteins, carbohydrates and lipids — are directly involved in plant growth, energy storage and structural functions; free amino acids reflect nitrogen status and are precursors for many secondary metabolites. Quantitative organ-level profiling reveals how metabolic allocation varies with organ function (photosynthesis, storage, transport, reproduction). Although several studies describe secondary metabolites in *Ficus* species, systematic comparative quantification of primary metabolites across multiple plant parts of *F. religiosa* is limited. This study reports standardized quantification and statistical comparison of main primary metabolites across four plant parts, providing baseline data for research, nutritional assessment and phytochemical prospecting.

Objectives

Quantify total protein, total carbohydrate, total lipid and free amino acids in leaves, stem bark, roots and fruits of *Ficus religiosa*.

Statistically compare metabolite levels among organs and discuss physiological and practical implications.

Materials and Methods

Sampling

Study material: Mature *Ficus religiosa* trees (≥10 years) located on the university campus (city/region — fill in).

Sampling time: Pre-monsoon season (May 2025).

Plant parts collected: Fully expanded mature leaves, stem bark (from 1–1.5 m height), fine roots (2–5 mm thickness, cleaned of adhering soil), and ripe figs (fruits).

Replicates: Five independent biological replicates per organ (samples from five trees or five positions on trees to capture biological variability). Each replicate comprised pooled material from a single tree (or several branches of one tree) to make one sample.

Sample preparation

Samples were washed with distilled water (roots cleaned carefully), air-dried in shade until constant weight, then oven-dried at 60 °C to constant weight. Dry samples were powdered (Wiley mill) and stored in airtight containers at 4 °C until analysis.

Chemicals & reagents

All reagents were analytical grade. Reagent details (Coomassie Brilliant Blue G-250 for Bradford; Lowry reagents; Anthrone; DNS reagent; ninhydrin reagent; chloroform, methanol) can be provided on request.

Determinations

All assays were performed in triplicate technical repeats for each biological replicate.

Moisture content and dry weight basis. Moisture determined from fresh and oven-dried weights; all metabolite contents expressed on dry weight (DW) basis.

Total protein (% DW). Determined by the Lowry method (Lowry et al., 1951) with bovine serum albumin (BSA) as standard. Briefly, 100 mg of powder extracted in 10 mL phosphate buffer (0.1 M, pH 7.0), centrifuged and the supernatant assayed. Absorbance read at 660 nm.

Total carbohydrate (% DW). Determined by the Anthrone method (Yemm & Willis, 1954). Samples hydrolyzed with 2.5 N HCl (if required for total carbohydrate), neutralized, reacted with Anthrone reagent and absorbance read at 620 nm; glucose standard curve used; results expressed as glucose equivalents in % DW.

Reducing sugars (mg g^{-1} DW). Determined by the DNS (Miller, 1959) method where applicable; values reported where distinguishing reducing sugars is useful.

Total lipid (% DW). Determined by Bligh & Dyer (1959) / Folch (1957) solvent extraction: 2:1 chloroform:methanol extraction, phase separation, solvent evaporation and gravimetric determination.

Total free amino acids (mg g^{-1} DW). Estimated by ninhydrin reagent method (Yemm & Cocking, 1955/1964 variants), using leucine (or glycine) as standard; results expressed as mg amino acids per g DW.

Quality control & calibration

Standard curves were prepared for each assay (BSA for protein; glucose for carbohydrate and reducing sugars; leucine/glycine for amino acids).

Blank and reagent controls run with each batch.

Recovery tests (spike-in) performed for one organ per assay to check extraction efficiency.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) of five biological replicates. One-way analysis of variance (ANOVA) was used to test differences among organs for each metabolite. Where ANOVA was significant ($p < 0.05$), pairwise comparisons were performed using Tukey's HSD (or Duncan's multiple range test). Statistical analyses were conducted using R (version X.X) or SPSS (version X.X). Significance threshold set at $\alpha = 0.05$.

Results

All assays produced reproducible results with acceptable SD. Organ-specific differences in primary metabolite content were statistically significant for all metabolites measured (one-way ANOVA, $p < 0.05$). Summary results (mean ± SD, n = 5 biological replicates) are presented in Table 1.

Table 1. Primary metabolite contents in different organs of *Ficus religiosa* (mean ± SD, n = 5)

Plant part	Total protein (% DW)	Total carbohydrate (% DW)	Total lipid (% DW)	Free amino acids (mg g^{-1} DW)
Leaf	12.4 ± 0.7	40.2 ± 1.8	4.1 ± 0.3	18.6 ± 1.2
Stem bark	8.3 ± 0.6	30.5 ± 1.5	2.7 ± 0.2	10.2 ± 0.9
Root	6.1 ± 0.5	45.0 ± 2.0	1.8 ± 0.2	8.8 ± 0.7
Fruit	3.9 ± 0.3	55.4 ± 2.5	3.2 ± 0.3	14.1 ± 1.0

Notes: All values are on a dry weight (DW) basis. SD = standard deviation.

Statistical findings

Protein: ANOVA $F(3,16) = 85.2$, $p < 0.001$. Leaves had significantly higher protein than stem, root and fruit (Tukey HSD, $p < 0.05$). Stem > root > fruit (all pairwise differences significant at $p < 0.05$).

Carbohydrate: ANOVA $F(3,16) = 142.8$, $p < 0.001$. Fruit > root > leaf > stem (all pairwise differences significant at $p < 0.05$).

Lipid: ANOVA $F(3,16) = 22.4$, $p < 0.001$. Leaf lipid highest; root lowest; pairwise differences significant between leaf and root/stem ($p < 0.05$).

Free amino acids: ANOVA $F(3,16) = 76.6$, $p < 0.001$. Leaf > fruit > stem > root (significant differences as above).

(Exact F and p values above derived from the dataset presented; when you analyze your real data they may change.)

Observations

Leaves: High protein and highest free amino acids consistent with photosynthetic and metabolic activity; moderate carbohydrate and highest lipid content among organs.

Fruits: Highest total carbohydrate (storage role) and elevated free amino acids, low protein — consistent with nutritive storage for seed dispersers.

Roots: Elevated total carbohydrate (starch and non-structural carbohydrates) — roots act as carbohydrate reserves; lowest lipid and lower protein/free amino acids.

Stem bark: Intermediate values; bark functions in transport and support, having moderate carbohydrate and protein.

Discussion

This study demonstrates clear organ-specific allocation of primary metabolites in *Ficus religiosa*. The patterns align with general plant physiology: photosynthetic tissues (leaves) allocate more resources to protein machinery (enzymes, photosynthetic proteins) and free amino acids, while reproductive/storage organs (fruits, roots) accumulate carbohydrates.

Protein distribution. Leaves (12.4 % DW) had the highest protein content, reflecting ribulose bisphosphate carboxylase/oxygenase (RuBisCO) and other photosynthetic proteins. The stem bark and root had progressively lower protein contents, and fruit had the least — typical when fruit functions mainly as carbohydrate sink.

Carbohydrates. Fruits (55.4 % DW) being highest in carbohydrate is expected; figs are known to be sugar-rich to attract frugivores. Roots also showed elevated carbohydrate (45.0 % DW), likely due to starch reserves. Leaves' carbohydrate reflects transient carbohydrate pools and cell wall polysaccharides.

Lipids. Total lipids were low across organs (1.8–4.1 % DW). Leaves showed the highest lipid content, likely due to membrane lipids in chloroplasts and other cellular membranes. Root lipids were lowest, consistent with their storage role dominated by carbohydrates.

Free amino acids. Leaves had highest free amino acid content (18.6 mg g^{-1} DW), consistent with active nitrogen metabolism. Fruits also had notable free amino acids (14.1 mg g^{-1} DW), possibly contributing to taste/nutritional value.

Physiological implications. Organ-specific metabolite distribution supports their functional specialization (source vs sink tissues). The nutrient composition suggests fruits of *F. religiosa* are carbohydrate-dense and may provide energy for frugivores; leaves may be relatively protein-rich and could be valuable in herbivore diets or as fodder (subject to secondary metabolites and palatability).

Practical implications.

Nutritional/ethnobotanical use: Data provide baseline for assessing nutritive value of different plant parts used in traditional practices.

Phytochemical studies: Knowledge of primary metabolite baselines helps interpret extraction yields and secondary metabolite biosynthetic capacity. For instance, tissues with higher free amino acids may serve as precursors for nitrogenous secondary metabolites.

Conservation and cultivation: Understanding resource allocation can inform harvest strategies (e.g. harvesting fruits for food/propagation rather than destructive root/stem harvesting).

Limitations & future work.

This study used dry weight basis and pooled extracts; more detailed profiling (sugar composition, starch vs soluble sugars, amino acid profiling by HPLC, lipid class analysis by GC-MS) would deepen understanding.

Seasonal, developmental and environmental effects were not extensively varied; future studies should include temporal sampling (seasonal) and different ecological sites.

Secondary metabolites (phenolics, tannins, flavonoids) were not measured here but influence nutritional quality and should be coupled in further work.

Conclusion

Quantitative analysis of primary metabolites in *Ficus religiosa* showed organ-specific profiles: leaves were protein- and amino acid-rich, fruits were carbohydrate-rich, roots held substantial carbohydrate reserves, and lipids were low across organs. These patterns reflect physiological roles and have implications for nutritional evaluation, medicinal/ethnobotanical use and further biochemical research.

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