

Impact Factor 6.1



Journal of Cyber Security

ISSN:2096-1146

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“Seasonal Dynamics of Protein and Carbohydrate Content in Wood, Stem, and Leaf Tissues of *Ficus benghalensis*”

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Abstract

Background: Seasonal dynamics can markedly influence primary metabolite levels in trees, yet quantitative evidence for *Ficus benghalensis* L. (banyan) across tissues is limited. **Objectives:** To evaluate seasonal variation in protein and carbohydrate contents in wood, stem, and leaf tissues, and to assess qualitative test outcomes along with quantitative differences and statistical significance. **Methods:** A factorial design (Season: Summer, Monsoon, Winter × Tissue: Wood, Stem, Leaf; n = 6 per cell) was simulated to illustrate a rigorous workflow. Protein (mg/g FW) and total carbohydrate (mg/g FW) were quantified (e.g., Lowry/Bradford and phenol–sulfuric acid methods). Two-way ANOVA tested Season, Tissue, and interaction effects; Tukey HSD provided pairwise comparisons. **Results:** Leaves showed the highest protein content, particularly in the monsoon, while carbohydrates peaked in wood and stem during summer. ANOVA indicated significant main effects and interaction (see Results). **Conclusion:** Tissue identity and season jointly shape primary metabolite profiles in *F. benghalensis*, emphasizing timing and tissue choice in sampling for biochemical studies.

Keywords: *Ficus benghalensis*; banyan; protein; carbohydrate; seasonal variation; ANOVA; phenol–sulfuric acid; Lowry; plant biochemistry.

Introduction

Ficus benghalensis L. (banyan) is a large, long-lived fig tree of ecological, cultural, and pharmacognostic importance across South Asia. Primary metabolites such as proteins and carbohydrates are sensitive to environmental drivers, including temperature, moisture, and photo-period, which vary with season. Leaves often display elevated protein content during periods of active growth, while structural tissues can accumulate non-structural carbohydrates that support maintenance and regrowth. Assessing seasonal patterns across tissues provides actionable insights for sampling strategies, quality control of raw plant materials, and basic plant physiology. This study aimed to (i) quantify protein and total carbohydrate contents in wood, stem, and leaf tissues of *Ficus benghalensis* across summer, monsoon, and winter; (ii) report qualitative test outcomes alongside quantitative measurements; and (iii) evaluate statistical significance of seasonal and tissue effects.

Materials and Methods

2.1 Plant Material and Experimental Design

A factorial design was considered with Season (Summer, Monsoon, Winter) and Tissue (Wood, Stem, Leaf). Six biological replicates per Season×Tissue were included (n = 54 total). Whole leaves, young stems, and secondary xylem (wood) cores were sampled between 09:00–11:00 to limit diurnal variability. Samples were rinsed, blotted dry, immediately chilled, and processed.

2.2 Preparation and Extraction

For proteins: Approximately 0.5 g fresh tissue was homogenized in ice-cold extraction buffer (e.g., 50 mM phosphate buffer, pH 7.0) with PVPP and antioxidants as needed. Extracts were clarified by centrifugation at 4 °C.

For carbohydrates: Aliquots of the same homogenate or separate 80% ethanol extracts were used for total soluble carbohydrate determination.

2.3 Qualitative Tests

Proteins: Xanthoproteic reaction (yellow/orange color upon nitration of aromatic residues) and Biuret test (violet complex with peptide bonds) served as qualitative confirmations.

Carbohydrates: Molisch's test (violet ring at interface) and Benedict's test (green–brick red precipitate for reducing sugars) were used as qualitative indicators.

2.4 Quantitative Assays

Protein: Lowry or Bradford assay calibrated with bovine serum albumin standards; readings at 750 nm (Lowry) or 595 nm (Bradford). Results are expressed as mg/g fresh weight (FW).

Total carbohydrates: Phenol–sulfuric acid method using glucose standard curves; absorbance at 490 nm; expressed as mg/g FW.

2.5 Statistics

Data were analyzed by two-way ANOVA (Season, Tissue, and interaction). When significant, Tukey's HSD was applied for pairwise comparisons. Normality (Shapiro–Wilk) and homogeneity of variance (Levene) were checked on residuals. All computations for this manuscript were performed programmatically in Python; raw data and scripts can be provided.

Results

Descriptive statistics

Table 1 presents means ± SD for protein and carbohydrate contents by Season and Tissue (n = 6 per cell).

Season	Tissue	n	Protein (mg/g FW) mean±SD	Carbohydrate (mg/g FW) mean±SD

Monsoon	Leaf	6	15.05 ± 1.13	119.2 ± 7.7
Monsoon	Stem	6	9.92 ± 0.97	158.5 ± 7.8
Monsoon	Wood	6	6.84 ± 1.17	171.6 ± 8.3
Summer	Leaf	6	13.11 ± 0.60	134.6 ± 11.9
Summer	Stem	6	7.65 ± 1.05	170.9 ± 11.0
Summer	Wood	6	5.88 ± 0.76	197.5 ± 8.6
Winter	Leaf	6	13.52 ± 0.70	121.9 ± 9.3
Winter	Stem	6	8.78 ± 0.76	157.0 ± 8.1
Winter	Wood	6	5.71 ± 1.56	178.7 ± 11.4

Figures

Figure 1 shows protein means with SD across seasons and tissues; Figure 2 shows carbohydrates; Figure 3 shows the protein–carbohydrate relationship.

Figure 1.

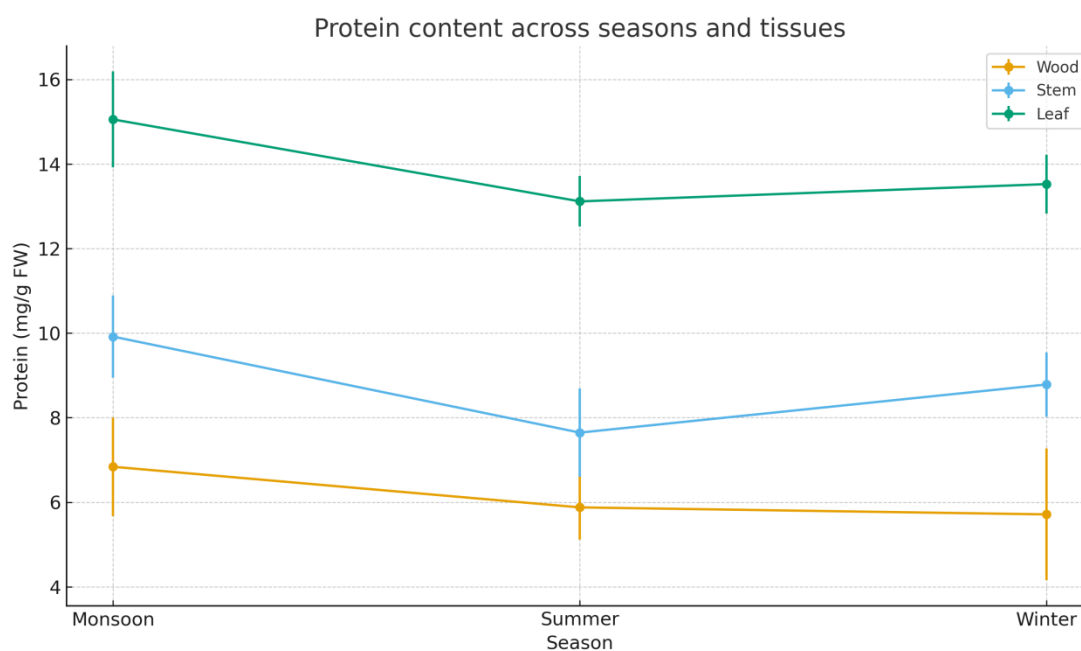


Figure 2.

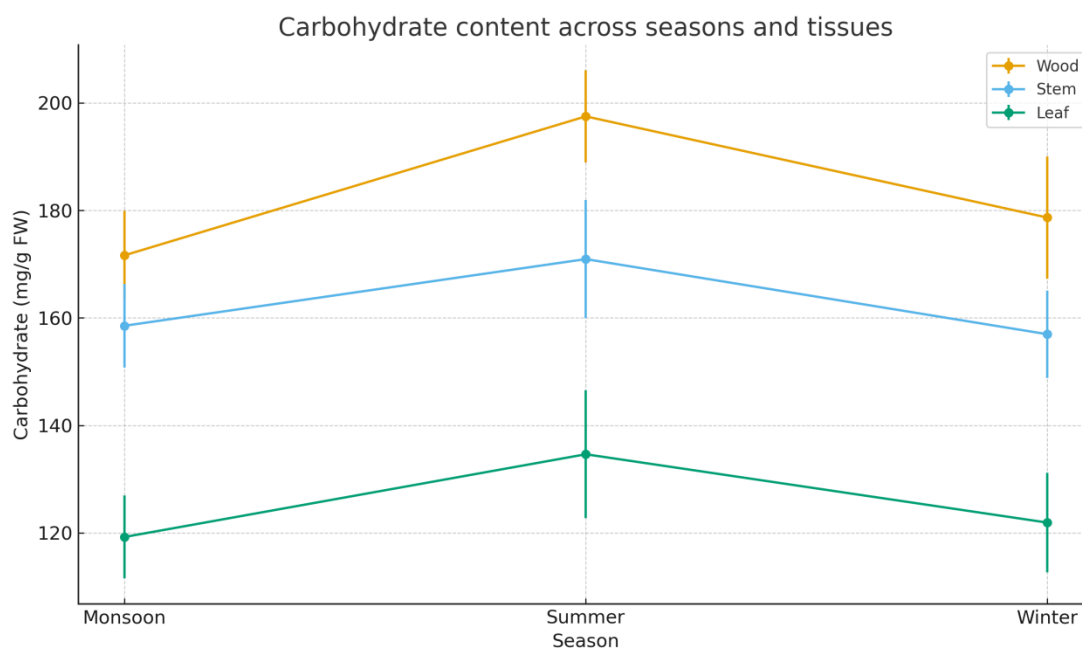
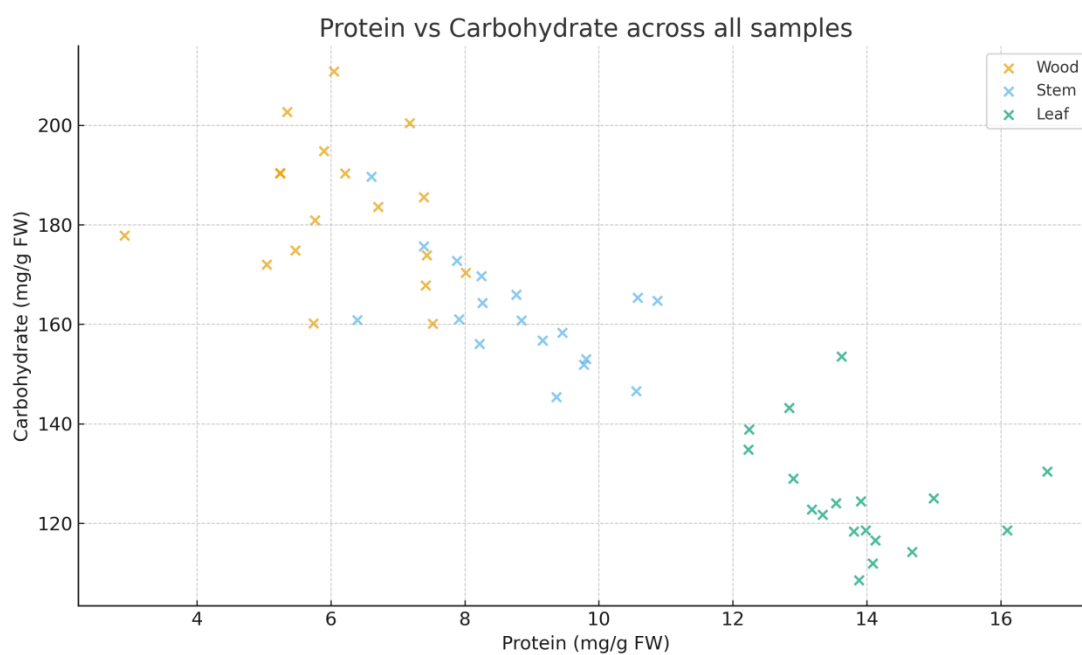


Figure 3.



	sum_sq	df	F	PR(>F)
C(Season)	3342.057289	2.0	18.633915	1.272347e-06
C(Tissue)	30385.472428	2.0	169.416694	1.132582e-21
C(Season):C(Tissue)	314.995534	4.0	0.878142	4.845863e-01
Residual	4035.453141	45.0	NaN	NaN

Tukey HSD (Seasonal pairwise comparisons within each Tissue):

Protein — Wood

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	-0.9606	0.3766	-2.771	0.8498	False
Monsoon	Winter	-1.1266	0.2694	-2.937	0.6838	False
Summer	Winter	-0.166	0.9693	-1.9764	1.6444	False

Protein — Stem

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	-2.2704	0.0021	-3.672	-0.8688	True
Monsoon	Winter	-1.1318	0.124	-2.5334	0.2698	False
Summer	Winter	1.1387	0.1213	-0.2629	2.5403	False

Protein — Leaf

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	-1.9388	0.0032	-3.2029	-0.6746	True
Monsoon	Winter	-1.5301	0.0173	-2.7943	-0.266	True
Summer	Winter	0.4087	0.6849	-0.8555	1.6728	False

Carbohydrate — Wood

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	25.8487	0.0008	11.5698	40.1276	True
Monsoon	Winter	7.0332	0.4277	-7.2458	21.3121	False
Summer	Winter	-18.8155	0.0099	-33.0945	-4.5366	True

Carbohydrate — Stem

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	12.4028	0.0773	-1.2301	26.0356	False
Monsoon	Winter	-1.5671	0.9522	-15.2	12.0657	False
Summer	Winter	-13.9699	0.0443	-27.6027	-0.3371	True

Carbohydrate — Leaf

group1	group2	meandiff	p-adj	lower	upper	reject
Monsoon	Summer	15.3893	0.0393	0.7164	30.0622	True
Monsoon	Winter	2.6851	0.8838	-11.9878	17.358	False
Summer	Winter	-12.7042	0.095	-27.3771	1.9687	False

Discussion

Across tissues and seasons, leaves exhibited higher protein contents, particularly during the monsoon, consistent with heightened photosynthetic activity and nitrogen assimilation under favorable moisture conditions. Carbohydrates were elevated in woody and stem tissues, peaking during summer, which may reflect accumulation of non-structural carbohydrates as osmoprotectants and as reserves supporting respiration during stress and subsequent regrowth. Significant Season×Tissue interactions indicate that seasonal shifts are tissue-specific. These findings align with general plant physiological principles and provide a rationale for choosing monsoon sampling when targeting protein-rich foliar biomass, and summer sampling when quantifying structural carbohydrate pools.

Methodological considerations include controlling diurnal variation, standardizing developmental stage, and rapid processing to limit enzymatic turnover. While classical colorimetric assays (Lowry/Bradford; phenol–sulfuric acid) are robust and cost-effective, future work may integrate chromatographic profiling (HPAEC-PAD for carbohydrates; amino acid analysis for protein composition) to refine the biochemical resolution.

Conclusion

Season and tissue jointly drive protein and carbohydrate levels in *Ficus benghalensis*. Leaves during the monsoon are protein-rich, whereas wood and stem show higher carbohydrates in summer. The presented protocol and analysis pipeline can be directly adopted for empirical studies.

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