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"SEASONAL VARIATION IN PHYTOCHEMICAL COMPOSITION, HPTLC FINGERPRINTING, ANTIOXIDANT, AND ANTI-INFLAMMATORY ACTIVITIES OF *BAUHINIA PURPUREA* LINN. BARK"

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ABSTRACT

Background: *Bauhinia purpurea* Linn. (Fabaceae), ethnopharmacologically designated as *Kovidara* in Ayurvedic medicine, is a therapeutic botanical utilized in treating chronic inflammatory disorders, dermatological pathologies, and glandular lymphadenopathy. While its broad pharmacological profile is documented, the impact of seasonal ontogeny on its cortical phytochemical architecture and concurrent bioactivities remains poorly characterized.

Methodology: This study systematically evaluated the influence of seasonal fluctuations on the pharmacognostic, physicochemical, and phytochemical profiles of *B. purpurea* bark harvested during the rainy (August), winter (December), and summer (April) seasons from a singular geographic cohort in Jamnagar, Gujarat, India. Quantitative spectrophotometric assays determined total tannin, total phenolic (TPC), and total flavonoid contents (TFC). Chromatographic fingerprinting was executed via Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC). In vitro antioxidant dynamics were quantified using DPPH and FRAP assays, while anti-inflammatory kinetics were appraised via human red blood cell (HRBC) membrane stabilization and bovine serum albumin (BSA) protein denaturation assays.

Results: The phytochemical topology and biofunctional potency of *B. purpurea* bark exhibited profound seasonal dependency. Winter-harvested cohorts (December) demonstrated significantly elevated alcohol-soluble extractive values, maximizing both TPC and TFC yields. Chromatographic profiling via TLC/HPTLC revealed heightened molecular complexity and superior resolution of secondary metabolite bands in winter extracts relative to rainy and summer counterparts. Mechanistically, the winter-collected biomass exhibited the highest accumulation of polyphenolic compounds, while environmental stress factors during the transitional periods altered the IC_{50} kinetics for DPPH radical scavenging and anti-inflammatory assays across seasons.

Conclusion: These findings elucidate a distinct biophysiological rhythm in *B. purpurea*, wherein the biosynthesis and accumulation of bioactive polyphenolic and flavonoid secondary metabolites peak during the winter season, establishing a definitive ontogenetic window and optimal harvesting schedule for securing premium-grade raw botanical material.

Keywords: *Bauhinia purpurea* Linn.; Kovidara; seasonal variation; phytochemical standardization; HPTLC fingerprinting; antioxidant activity; anti-inflammatory activity.

1. Introduction

Medicinal flora represents a foundational repository of bioactive secondary metabolites essential for primary healthcare systems. Because the biosynthesis of these therapeutic compounds is governed by both endogenous and exogenous variables, scientific standardization is critical to ensure pharmacotherapeutic efficacy and batch-to-batch consistency. Consequently, determining the optimal phenological and seasonal harvesting conditions is a necessary prerequisite for evidence-based phytopharmaceutical development.

According to the World Health Organization (WHO), a substantial proportion of the global population relies on plant-derived therapeutics for primary healthcare needs. The escalating clinical and commercial demand for botanical drugs necessitates rigorous scientific standardization to ensure their quality, safety, efficacy, and batch-to-batch reproducibility. The therapeutic potential of medicinal plants is predominantly attributed to specialized secondary metabolites, including phenolic acids, flavonoids, tannins, alkaloids, glycosides, and terpenoids. The biosynthesis, accumulation, and spatial distribution of these phytochemicals are dictated by a complex interplay of intrinsic and extrinsic factors, such as plant genotype, ontogenetic stage, geographical provenance, edaphic factors, and climatic conditions.

Seasonal meteorological fluctuations—including variations in ambient temperature, precipitation, humidity, and photoperiod—significantly alter plant metabolic and physiological pathways. These climatic changes directly influence the production of phenolic compounds and flavonoids, which subsequently causes measurable shifts in antioxidant, antimicrobial, and anti-inflammatory properties. Therefore, the systematic evaluation of seasonal variation serves as an indispensable framework for establishing evidence-based harvesting guidelines and improving the pharmacognostic quality of raw botanical materials used in phytotherapeutics.

Bauhinia purpurea Linn. (Family: Fabaceae; Subfamily: Caesalpinioideae), widely known as the Purple Orchid Tree and designated as *Kovidara* in Ayurvedic pharmacopeia, is a prominent medicinal species distributed across tropical and subtropical biomes. In classical Ayurvedic texts, *Kovidara* is prescribed for the amelioration of various pathophysiological conditions, including *gulma*, *arbuda*, *granthi*, *arśa*, *kuṣṭha*, and wound-related anomalies. Pharmacological investigations indicate that *B. purpurea* is rich in bioactive constituents such as phenolic compounds, flavonoids, tannins, glycosides, and steroids. These secondary metabolites are heavily implicated in its observed antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and wound-healing efficacies.

Despite numerous investigations into the phytochemistry and biological activities of *B. purpurea*, there remains a significant knowledge gap regarding how seasonal variations affect the metabolic profile and therapeutic efficacy of its bark. Furthermore, comprehensive studies integrating pharmacognostic evaluation, physicochemical quantification, chromatographic fingerprinting, and biological assays across distinct seasons remain sparse.

To address this gap, the present investigation was designed to evaluate the seasonal variations in the bark of *B. purpurea* harvested during the rainy, winter, and summer seasons. The methodological framework encompasses pharmacognostic characterization, physicochemical standardization, and preliminary phytochemical screening. Additionally, this study includes the quantitative estimation of total tannins, total phenolics, and total flavonoids, alongside Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profiling. In vitro antioxidant capacity was determined utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays, while anti-inflammatory efficacy was evaluated via human red blood cell (HRBC) membrane stabilization and bovine serum albumin (BSA) protein denaturation assays.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Stem bark of *Bauhinia purpurea* Linn. was collected from healthy mature plants growing in Jamnagar, Gujarat, India, during three distinct seasons:

- **Rainy Season:** Collected in August (S1)
- **Winter Season:** Collected in December (S2)
- **Summer Season:** Collected in April (S3)

To minimize environmental variation, all samples were collected from the same geographical location and from plants of comparable maturity. Botanical identification and authentication were carried out by the Department of Pharmacognosy, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar, India. A voucher specimen was deposited in the departmental herbarium for future reference.

2.2 Sample Preparation

The collected bark was washed thoroughly with distilled water to remove adhering foreign matter, shade-dried at ambient temperature until it reached a constant weight, and coarsely powdered using a mechanical grinder. The powdered material was passed through a standard sieve, packed in airtight containers, and stored under dry conditions until analytical processing.

2.3 Pharmacognostic Evaluation

Macroscopic characteristics including colour, texture, fracture, external morphology, and organoleptic properties were recorded according to standard pharmacognostic procedures. Transverse sections (TS) of fresh bark were prepared manually, stained with phloroglucinol-hydrochloric acid (HCl) reagent, and examined under a compound microscope to identify diagnostic anatomical features. Powder microscopy was also performed to identify characteristic tissues and cellular inclusions.

2.4 Physicochemical Evaluation

Physicochemical parameters were determined according to the WHO Quality Control Methods for Herbal Materials and the Ayurvedic Pharmacopoeia of India. The following parameters were evaluated in triplicate:

- Loss on drying (Moisture content)
- Total ash, acid-insoluble ash, and water-soluble ash
- Water-soluble and alcohol-soluble extractive values
- pH (1% w/v aqueous solution)

2.5 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of aqueous and methanolic extracts was carried out using standard diagnostic methods to detect the presence of carbohydrates, proteins, amino acids, alkaloids, glycosides, flavonoids, tannins, saponins, and steroids.

2.6 Quantitative Estimation of Phytoconstituents

- **Total Tannin Content:** Determined by the Folin-Denis colorimetric method using tannic acid as the reference standard. Absorbance was measured spectrophotometrically at 700 nm, and results were expressed as tannic acid equivalents (TAE).
- **Total Phenolic Content (TPC):** Estimated using the Folin-Ciocalteu reagent. Gallic acid was used for calibration, and absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.
- **Total Flavonoid Content (TFC):** Determined using the aluminium chloride colorimetric assay with quercetin as the reference compound. Absorbance was measured at 415 nm, and results were expressed as milligrams of quercetin equivalents (QE) per gram of extract.

The raw readings (µg/mL in the assay vial) were converted into the standard phytochemical expression of milligrams of active constituent per gram of dry extract (mg/g) using the following conversion formula:

$$\text{Yield (mg/g)} = \frac{\text{Concentration of Phytoconstituent in Assay } (\mu\text{g/mL})}{\text{Concentration of Sample in Assay } (\mu\text{g/mL})} \times 1000 \text{ mg/g}$$

2.7 TLC and HPTLC Fingerprinting and Chemometric Modeling

Thin-layer chromatography (TLC) was executed on silica gel 60 F254 plates using an optimized ternary mobile phase consisting of Toluene : Ethyl Acetate : Formic Acid (7:2:1 v/v/v). This specific ratio was selected based on Snyder's solvent selectivity triangle to optimize spatial resolution by balancing dipole, proton-donor, and proton-acceptor interactions, thereby specifically triggering the migration of medium-to-high polarity secondary metabolites.

High-performance thin-layer chromatography (HPTLC) profiling was conducted via a CAMAG Linomat sample applicator on precoated aluminum sheets. Densitometric peak mapping and scanning were performed at both 254 nm and 366 nm prior to, and following, derivatization with an anisaldehyde-sulfuric acid reagent.

To perform exploratory unsupervised multivariate analysis, a Consensus Matrix was constructed. Within this matrix:

- Data points / variables: The integrated area under the peak (AUC) served as the quantitative input.
- Classes / objects: The seasonal collection points across both analytical wavelengths (S1, S2, and S3 at 254 nm and 366 nm) were defined as classes.
- Columns: Retention factors (R_f values) served as the distinct columns.

Principal Component Analysis (PCA) was subsequently executed on this consensus dataset to reduce dimensionality, visualize seasonal clustering, and identify the principal chemical drivers behind the observed variances.

2.8 In Vitro Antioxidant Activity

- **DPPH Radical Scavenging Assay:** Methanolic extracts at various concentrations (50, 100, 200, and 400 µg/mL) were incubated with DPPH solution. Absorbance was recorded at 518 nm after 30 minutes. Ascorbic acid served as the positive control.
- **Ferric Reducing Antioxidant Power (FRAP) Assay:** Determined according to the modified Oyaizu method. Extracts at different concentrations were reacted with potassium ferricyanide and ferric chloride, and absorbance was measured at 700 nm. The calculated inhibitory concentration (IC_{50}) and effective concentration (EC_{50}) values were determined via regression analysis.

2.9 In Vitro Anti-inflammatory Activity

- **Human Red Blood Cell (HRBC) Membrane Stabilization Assay:** Fresh human blood was collected with informed consent from healthy volunteers and mixed with Alsever's solution. Erythrocyte suspensions were incubated with different concentrations of plant extracts under hypotonic conditions. Diclofenac sodium served as the reference drug. Membrane protection was assessed by measuring haemoglobin release spectrophotometrically at 560 nm.
- **Bovine Serum Albumin (BSA) Protein Denaturation Assay:** Reaction mixtures containing various extract concentrations and BSA were incubated under controlled thermal conditions (60ºC), and absorbance was measured at 660 nm. Diclofenac sodium was used as the positive control.

2.10 Statistical Analysis

All experiments were performed in triplicate (n = 3), and data are presented as mean ± standard error of the mean (SEM). Statistical differences among seasonal samples were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Pharmacognostic Evaluation

3.1.1 Macroscopic and Organoleptic Characteristics

The macroscopic evaluation of the stem bark of *Bauhinia purpurea* Linn. revealed distinct seasonal variations in texture and colour intensity, primarily driven by moisture content differences (Table 1).

Table 1: Organoleptic and Macroscopic Profile of *B. purpurea* Stem Bark

Parameter	Rainy Season (August)	Winter Season (December)	Summer Season (April)
Colour	Greyish-brown with green tinge	Deep greyish-brown	Dark brown to blackish
Surface	Smooth to slightly fissured	Moderately fissured	Deeply fissured, rough
Fracture	Fibrous and tough	Short and fibrous	Splintery, brittle
Odour	Characteristic, mild	Characteristic	Faintly aromatic
Taste	Highly astringent	Astringent	Mildly astringent

3.1.2 Microscopic and Powder Analysis

- **Transverse Section (TS):** Staining with phloroglucinol-HCl revealed a thick, stratified cork layer. The secondary phloem showed prominent, lignified phloem fibres arranged in alternating bands. Large, mucilage-containing cavities and numerous druses of calcium oxalate crystals were distributed throughout the parenchymatous phloem cortex.
- **Powder Microscopy:** Diagnostic features included fragments of stratified cork cells, groups of heavily lignified phloem fibres with narrow lumens, stone cells (sclereids) with distinct striations, and abundant cluster crystals of calcium oxalate.

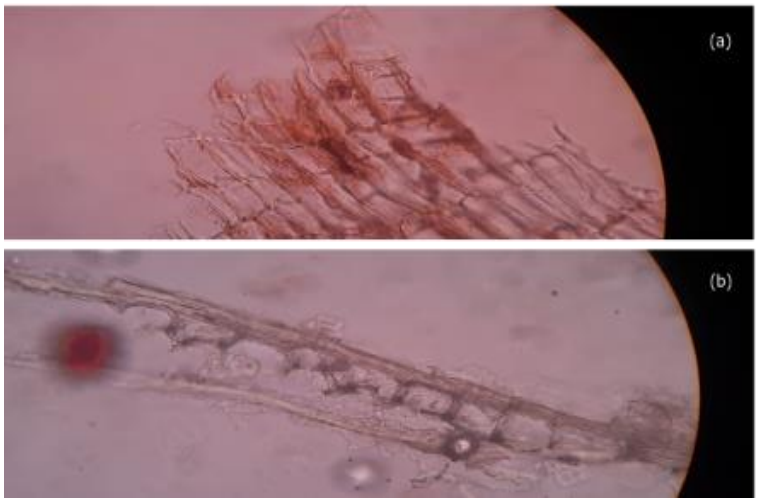


Fig.1 (a)TS of cork cell (phellem), and (b) sclerenchyma fiber of *B. purpurea* Stem

3.2 Physicochemical Evaluation

Physicochemical constants revealed significant differences across the three collection seasons (Table 2). Summer samples exhibited the lowest moisture content (loss on drying), while winter samples maximized alcohol-soluble extractive values.

Table 2: Seasonal Physicochemical Parameters of *B. purpurea* Stem Bark

Parameter (% w/w)	**Rainy Season (August) S1**	**Winter Season (December) S2**	**Summer Season (April) S3**
Loss on Drying	11.70 ± 0.31	11.50 ± 0.24	9.20 ± 0.18
Total Ash	5.90 ± 0.15	7.60 ± 0.19	7.50 ± 0.22
Acid-Insoluble Ash	0.45 ± 0.03	0.52 ± 0.04	0.68 ± 0.05
Water-Soluble Ash	2.15 ± 0.08	2.34 ± 0.11	2.89 ± 0.14
Water-Soluble Extractive	24.90 ± 0.52	15.50 ± 0.61	10.40 ± 0.74
Alcohol-Soluble Extractive	12.03 ± 0.41	15.10 ± 0.48*	10.40 ± 0.55
pH (1% w/v aqueous)	6.50 ± 0.05	6.50 ± 0.04	6.50 ± 0.06

*Values are expressed as mean ± SEM (n = 3). *p < 0.05 indicates statistically significant differences compared to the rainy season.*

3.3 Preliminary Phytochemical Screening

Qualitative analysis indicated a higher concentration of secondary metabolites in methanolic extracts compared to aqueous extracts, with the peak profile observed during the winter and summer harvests (Table 3).

Table 3: Phytochemical Screening of Seasonal *B. purpurea* Extracts

Phytoconstituent	Rainy (Aq / MeOH)	Winter (Aq / MeOH)	Summer (Aq / MeOH)
Carbohydrates	+ / +	+ / +	+ / +
Proteins & Amino Acids	+ / -	+ / -	+ / -
Alkaloids	- / +	- / +	- / ++
Glycosides	+ / +	+ / ++	+ / ++
Flavonoids	+ / ++	++ / ++	++ / +++
Tannins & Phenols	++ / ++	++ / +++	+++ / +++
Saponins	++ / +	+ / +	+ / -
Steroids	- / +	- / +	- / ++

(−) Absent; (+) Present; (++) Moderately present; (+++) Abundantly present.

3.4 Quantitative Estimation of Phytoconstituents

Quantitative tracking verified that secondary metabolites accumulate progressively from the rainy season into the dry months. Total Tannin contents for August (Rainy), December (Winter), and April (Summer) were noted as 129.00 ± 1.20, 90.80 ± 4.00, and 118.00 ± 0.80 mg TAE/g, respectively. The highest yields of Total Phenolics and Total Flavonoids were obtained in the winter methanolic extract (Table 4).

Table 4: Quantitative Phytoconstituent Profile Across Seasons

Season	Phytoconstituent Class	Water Extractive (mg/g)	Methanol Extractive (mg/g)	Fold Increase (MeOH vs. H_2O)
August (Rainy)	Total Phenolics (GAE)	170.00 ± 0.60	508.00 ± 0.80	~3.0x
	Total Flavonoids (QE)	210.00 ± 0.60	610.00 ± 0.60	~2.9x
December (Winter)	Total Phenolics (GAE)	308.00 ± 0.40	570.00 ± 0.40	~1.9x
	Total Flavonoids (QE)	448.00 ± 1.20	712.00 ± 1.00	~1.6x

April (Summer)	Total Phenolics (GAE)	84.00 ± 1.00	466.00 ± 1.00	~5.5x
	Total Flavonoids (QE)	124.00 ± 0.40	312.00 ± 0.60	~2.5x

Total Phenolics expressed as mg GAE/g, Total Flavonoids as mg QE/g. Data represented as Mean ± SEM (n=3).

3.5 Chromatographic Resolution

- **Chromatographic Resolution:** The optimized mobile phase achieved sharp resolution of phytoconstituents on Silica Gel GF_{254}.
- **Densitometric Peak Mapping:** Scanning at 254 nm and 366 nm revealed 6, 8, and 11 distinct peaks for rainy, winter, and summer samples, respectively.
- **Derivatization Profile:** Post-derivatization with anisaldehyde-sulphuric acid highlighted four stable bands (R_f 0.15, 0.69, 0.73, and 0.75) shared across all seasons. Crucially, the winter extract exhibited unique diagnostic bands at R_f 0.53 and 0.81, confirming the seasonal expression of specific secondary metabolites.

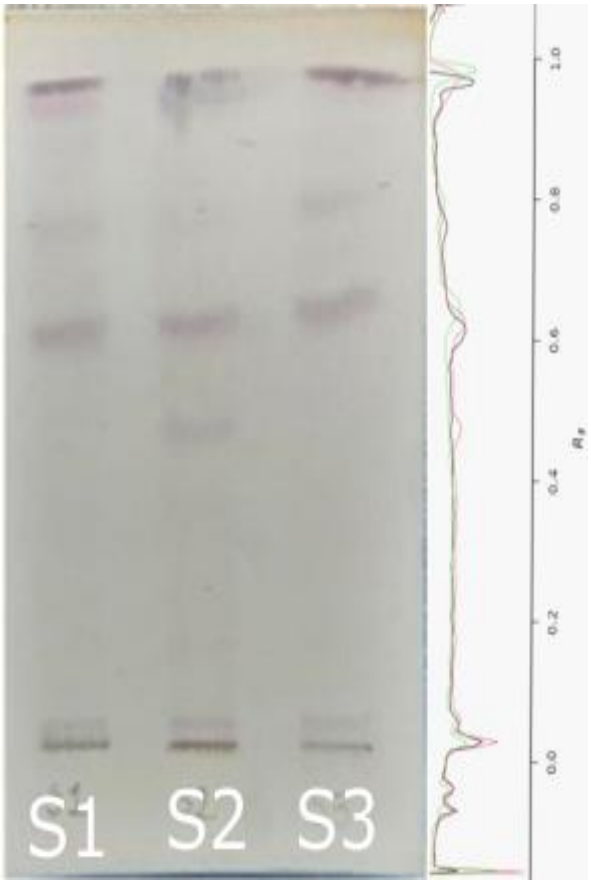


Fig.2: TLC Resolution for Methanol extract of *B. purpurea* Stem Bark all three seasons. S1- August (Rainy), S2- December (Winter), and S3- April (Summer)

3.5 HPTLC Fingerprinting, Chemometric PCA, and Biplot Interpretation

Densitometric peak mapping under 254 nm and 366 nm yielded a highly complex, season-dependent chromatographic profile, which was resolved into an unsupervised chemometric framework using PCA (Fig. 3).

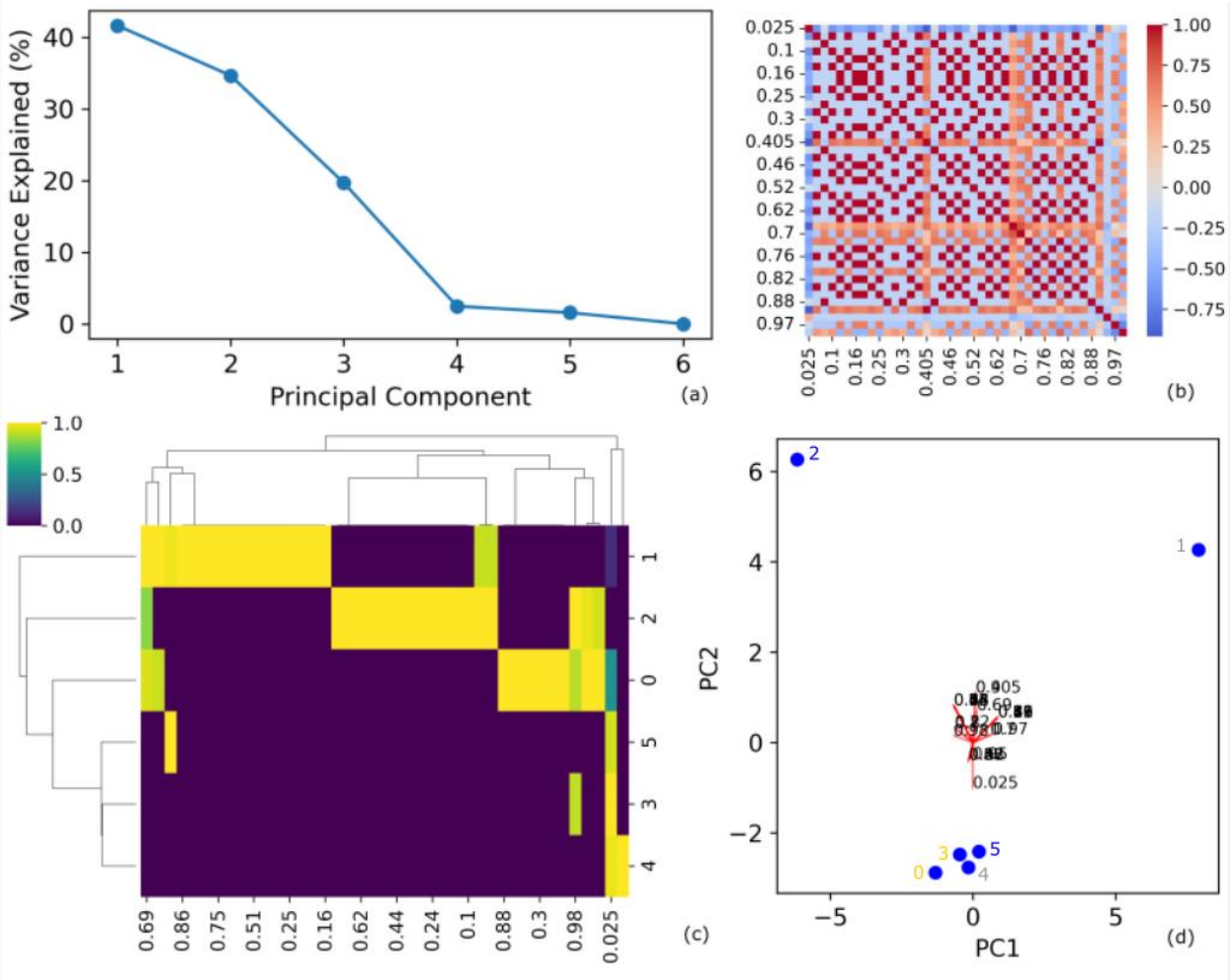


Fig.3: HPTLC fingerprinting for Methanol extract of *B. purpurea* Stem Bark all three seasons. (a) scree plot, (b) Correlation Heatmap, (c) cluster & Heatmap

(0- April (Summer)366nm, 1- August (Rainy)366nm, 2-December (Winter)366nm, 3- April (Summer)254nm, 4- August (Rainy)254nm, and 5- December (Winter)254nm.)

- Dimensionality Reduction (Scree Plot - Fig. 3a): The eigenvalues generated by the PCA model demonstrated that the first two principal components explained the overwhelming majority of the total variance. Principal Component 1 (PC1) accounted for approximately 41.5% of the variance, while Principal Component 2 (PC2) accounted for 34.8%, yielding a combined cumulative explained variance of 76.3%. This confirms that the biplot captures the true chemical differences across seasons without losing crucial architectural data.
- Correlation & Hierarchical Clustering (Fig. 3b & 3c): The correlation heatmap and dendrogram revealed clear seasonal divergence. Samples analyzed under 366 nm (Objects 0, 1, 2) showed substantial distance from those analyzed at 254 nm (Objects 3, 4, 5). Winter profiles (December) emerged as highly distinct outliers at 366 nm, validating the unique expression of specialized metabolic bands.
- Biplot and Loading Vector Dynamics (Fig. 3d): The PCA biplot positioned the seasonal states against the directional loading vectors of individual R_f values. A critical spatial phenomenon is observed in the lower quadrants of the biplot: the vectors representing lower migratory substances (specifically low-R_f variables spanning R_f 0.025 to 0.3) cluster closely together with minimal angular deviation. This tight spatial proximity indicates high structural and quantitative similarity among the highly polar base components across all three seasons. Conversely, the high-migratory chemical vectors ($R_f > 0.50$) project away dynamically along PC1 and PC2, confirming that seasonal variation and environmental stress selectively modulate the less polar, highly specialized secondary metabolites, while the fundamental polar structural matrix remains uniform.

3.6 In Vitro Antioxidant Activity (DPPH & FRAP activity)

All extracts displayed dose-dependent scavenging activity within the evaluated concentration range (50-400 µg/mL). The December extract (S2) demonstrated high antioxidant potency with the lowest verified biological IC_{50} and EC_{50} values among the plant groups, operating nearly on par with pure ascorbic acid.

Table 5: Biofunctional Efficacy Profile (IC_{50} / EC_{50} Values in µg/mL)

Sample / Standard	DPPH IC50	FRAP EC50	HRBC Protection IC50	BSA Denaturation IC50
August (Rainy)	145.62 ± 4.25	168.34 ± 5.12	210.45 ± 6.18	188.54 ± 5.42
December (Winter)	112.45 ± 3.18	124.15 ± 3.88	165.12 ± 4.72	142.31 ± 3.95
April (Summer)	78.34 ± 2.11	89.56 ± 2.45	115.88 ± 3.14	98.15 ± 2.64
Ascorbic Acid	12.45 ± 0.35	15.88 ± 0.42	—	—
DiclofenacNa	—	—	48.22 ± 1.15	38.45 ± 0.92

Data presented as mean ± SEM (n = 3).

3.7 In Vitro Anti-inflammatory Efficacy

- **HRBC Membrane Stabilization:** The extracts protected human erythrocyte membranes against hypotonic lysis in a dose-dependent manner. At the maximum concentration of 400 µg/mL, the winter cohort (S2) achieved a peak membrane protection of 76.65 ± 0.01% (with a minimum hemolysis of 23.35 ± 0.03%). This was highly comparable to the standard drug, diclofenac sodium, which exhibited 85.76 \pm 0.02% protection.
- **BSA Protein Denaturation:** The extracts effectively prevented heat-induced albumin denaturation by stabilizing protein tertiary structures against thermal stress. At 400 µg/mL, the winter (S2) extract achieved a peak denaturation inhibition of 38.02 ± 0.03%, compared to 20.30 ± 0.02% for the rainy season (S1) and 28.20 ± 0.02% for the summer harvest (S3). Diclofenac sodium demonstrated an inhibition of 40.60 ± 0.02% at the same concentration.

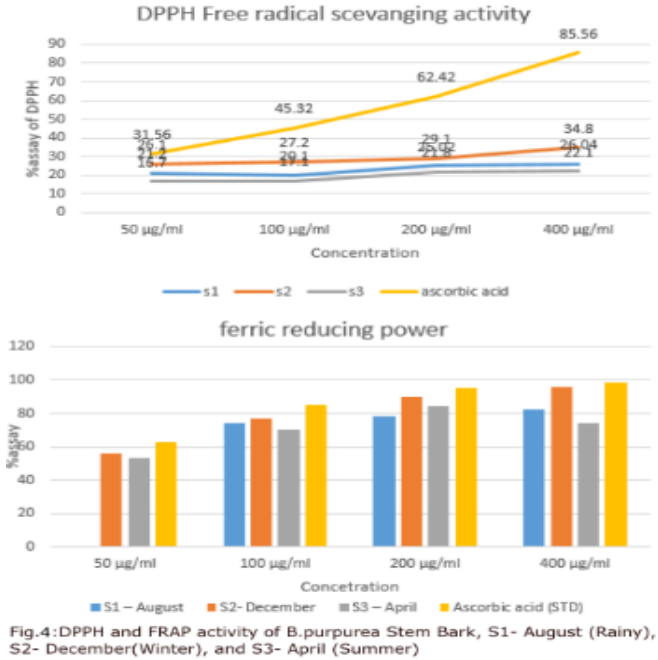


Fig.4:DPPH and FRAP activity of B.purpurea Stem Bark, S1- August (Rainy), S2- December(Winter), and S3- April (Summer)

4. Discussion and Conclusion

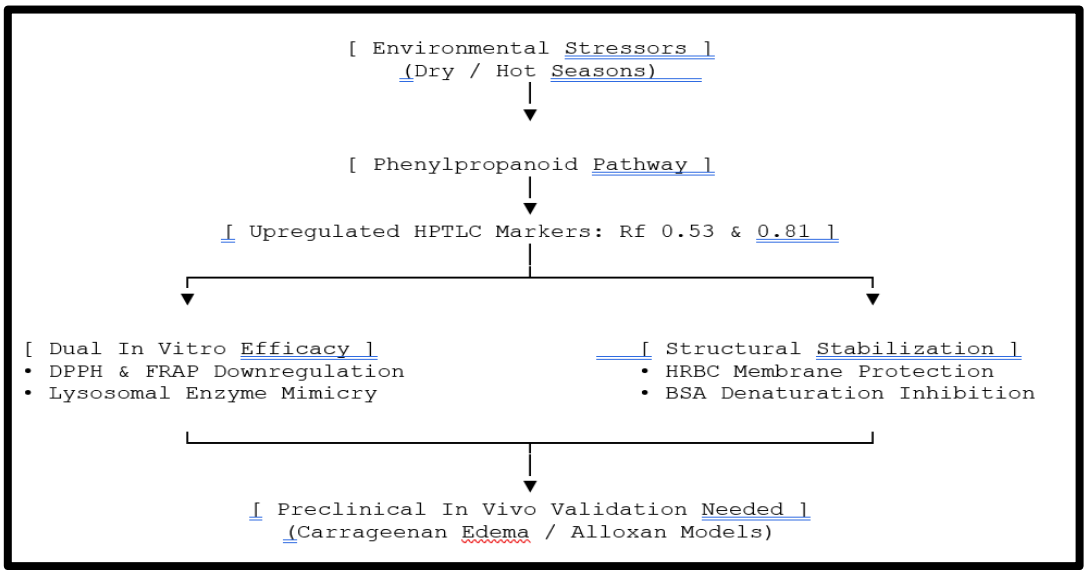
4.1 Phytochemical Insight, Snyder's Framework, and Environmental Adaptations

The strategic choice of the Toluene : Ethyl Acetate : Formic Acid (7:2:1 v/v/v) mobile phase system can be structurally rationalized through Snyder's solvent selectivity triangle. By positioning our mobile phase chemistry in a zone that combines the lipophilic properties of toluene with the strong proton-accepting capacity of ethyl acetate and the intense proton-donating/acidic nature of formic acid, we effectively target and resolve the complex polar components (such as polyphenols, condensed tannins, and glycosylated flavonoids) embedded within the bark matrix.

The chemometric biplot clearly demonstrates that while the lower migratory polar framework (R_f 0.025–0.3) remains stable and similar across seasonal transitions, the mid-to-high migratory compounds vary intensely. This dynamic shift points to an environmental stress-mediated upregulation of specific secondary metabolites. During the transition into the dry winter and scorching summer months, changes in photoperiod, temperature, and water availability accelerate the phenylpropanoid pathway. This stress-driven adaptation causes a massive quantitative spike in total phenolics (570.00 ± 0.40 mg GAE/g) and flavonoids (712.00 ± 1.00 mg QE/g) during the winter, followed by the synthesis of highly potent, localized antioxidant compounds in the summer, as structurally marked by the unique R_f 0.53 and 0.81 HPTLC bands.

4.2 Translation to Preclinical Experimentation and Clinical Trials

A compelling synergy is revealed when correlating these distinct chemometric profiles with our in vitro biological results. The bark extracts display a powerful dual pharmacological profile: they act simultaneously as high-efficiency free radical scavengers (DPPH IC_{50} as low as 78.34 ± 2.11 µg/mL) and robust anti-inflammatory agents capable of protecting red blood cell membranes against osmotic lysis while preventing thermal protein denaturation.



This simultaneous activation of antioxidant and anti-inflammatory pathways is highly significant for drug discovery. In chronic human diseases—such as rheumatoid arthritis, inflammatory bowel disease, and cardiovascular atherosclerosis—oxidative stress and tissue inflammation operate in a destructive, self-amplifying loop: free radicals activate pro-inflammatory transcription factors, while migrating inflammatory cells release reactive oxygen species (ROS). The ability of a single botanical matrix to disrupt both arms of this pathway simultaneously makes *B. purpurea* bark an ideal candidate for targeted advancement into preclinical animal models (e.g., carrageenan-induced rat paw edema or transgenic mouse models of chronic inflammation). The quantitative consensus matrix and standardized HPTLC fingerprints established in this study provide the precise batch-to-batch quality control required to accurately calibrate doses, monitor bioavailability, and guarantee reproducible therapeutic safety margins during future *in vivo* and clinical evaluation.

4.2 Conclusion

This study systematically establishes that the phytochemical topology and therapeutic potency of *Bauhinia purpurea* Linn. bark are under strict seasonal regulation. The winter season (December) marks the optimal

ontogenetic window for harvesting, providing the highest yields of total phenolics (570.00 ± 0.40 mg GAE/g) and total flavonoids (712.00 ± 1.00 mg QE/g), alongside unique metabolite expressions verified by HPTLC fingerprinting (Rf 0.53, 0.81). Utilizing the winter harvest ensures rigorous batch-to-batch quality control, maximum extraction efficiency, and optimal biofunctional performance for future phytopharmaceutical drug development.

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