

Quantitative Assessment of Phenolic and Flavonoid Metabolites and their Contribution to Antioxidant Capacity in Selected Medicinal Plants of North Bihar

Journal- Research & Review: Journal of Botany
ISSN- 2278-2222, Volume 15, Issue-2, year- 2026

Received 25/09/2026

Accepted 03/10/2026

Article Type- Research

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Abstract

Introduction: Medicinal plants remain integral to traditional healthcare systems across India, with North Bihar harboring a rich diversity of ethnobotanically significant species. However, systematic quantitative data on the phytochemical profiles and antioxidant potential of these plants from this specific geographical region remain limited. **Objective:** This study aimed to quantitatively assess the total phenolic content (TPC), total flavonoid content (TFC), and in vitro antioxidant capacity of three medicinally important plants—*Azadirachta indica*, *Ocimum sanctum*, and *Tinospora cordifolia*—collected from three districts of North Bihar: Darbhanga, Madhubani, and Sitamarhi. **Methods:** Plant materials were collected from the selected study sites in North Bihar and taxonomically authenticated prior to laboratory analysis. The dried samples were extracted using a 70% hydroethanolic solvent (ethanol:water, 70:30 v/v) to obtain phytochemical-rich extracts. Total phenolic content (TPC) was quantified by the Folin–Ciocalteu colorimetric method, whereas total flavonoid content (TFC) was estimated using the aluminium chloride colorimetric assay. The antioxidant potential of each extract was investigated through DPPH free-radical scavenging, ABTS radical cation decolorization, and Ferric Reducing Antioxidant Power (FRAP) assays. To examine variations among species and collection sites, the experimental data were further analysed using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), enabling the identification of phytochemical and antioxidant relationships. **Results:** Significant variation in phytochemical content and antioxidant capacity was observed across species and collection sites. *Tinospora cordifolia* consistently exhibited the highest TPC (42.36 ± 1.45 to 58.72 ± 1.82 mg GAE/g) and TFC (28.14 ± 0.92 to 39.65 ± 1.23 mg QE/g), followed by *Ocimum sanctum* and *Azadirachta indica*. The DPPH IC₅₀ values ranged from 32.45 ± 1.23 µg/mL (*T. cordifolia*, Madhubani) to 78.36 ± 2.14 µg/mL (*A. indica*, Sitamarhi). Strong positive correlations ($R^2 > 0.85$) were established between phenolic/flavonoid content and antioxidant capacity. PCA explained 94.68% of total variance, clearly discriminating species and geographical clusters. **Conclusion:** The findings validate the traditional therapeutic uses of these plants and highlight the influence of geographical location on bioactive compound accumulation. *Tinospora cordifolia* from Madhubani emerged as the most promising source of natural antioxidants.

Keywords: Antioxidant activity, *Azadirachta indica*, Flavonoids, North Bihar, *Ocimum sanctum*, Phenolic compounds, Principal Component Analysis, *Tinospora cordifolia*

1. Introduction

The use of medicinal plants as therapeutic agents has been an integral part of human healthcare since ancient times. In India, the Ayurvedic tradition has preserved extensive knowledge of the

healing properties of numerous plant species over thousands of years. Increasingly, contemporary scientific research is providing experimental evidence that supports many of these traditional medicinal applications. Medicinal plants are rich reservoirs of bioactive secondary metabolites, among which phenolic compounds and flavonoids are particularly noteworthy due to their potent antioxidant properties and diverse pharmacological activities. Reactive oxygen species (ROS) and other free radicals are implicated in the pathogenesis of numerous degenerative diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions. Natural antioxidants derived from plant sources offer a safer alternative to synthetic antioxidants, which are often associated with adverse health effects. The quantification of phenolic and flavonoid metabolites in medicinal plants is therefore essential for understanding their therapeutic potential and developing evidence-based herbal formulations[1]. North Bihar, comprising districts such as Darbhanga, Madhubani, and Sitamarhi, is characterized by its alluvial soil composition, humid subtropical climate, and rich agricultural heritage. This region supports diverse medicinal plant species traditionally used by local communities. However, the systematic quantitative assessment of phytochemical constituents and antioxidant properties of medicinal plants from this specific geographical region remains inadequately documented. Previous studies have demonstrated that environmental factors including soil composition, temperature, rainfall, and altitude significantly influence secondary metabolite accumulation[2].

Azadirachta indica (Neem, Meliaceae) is a versatile medicinal tree extensively used in traditional medicine for its antibacterial, antifungal, anti-inflammatory, and immunomodulatory properties. The plant contains numerous bioactive compounds including nimbin, nimbolide, and various phenolic constituents.

Ocimum sanctum (Tulsi; family Lamiaceae) is one of the most valued medicinal herbs in India, holding both cultural and therapeutic significance. Its pharmacological potential is largely associated with bioactive constituents such as eugenol, ursolic acid, rosmarinic acid, and a diverse group of flavonoids. These compounds contribute to its well-documented adaptogenic, antimicrobial, anti-inflammatory, and antioxidant properties. Members of the Lamiaceae family are widely recognized for their abundant phenolic metabolites, making them important natural sources of antioxidant phytochemicals[3].

Tinospora cordifolia (Giloy, Menispermaceae) is a climbing shrub renowned for its immunomodulatory, antipyretic, antidiabetic, and hepatoprotective properties. Its stem contains diverse bioactive compounds including alkaloids, terpenoids, lignans, and phenolic glycosides with significant antioxidant potential. While individual studies have investigated the phytochemical profiles of these plants from various geographical regions, comparative quantitative data from North Bihar's distinct agro-climatic zones remain scarce. Furthermore, the influence of geographical location on secondary metabolite accumulation in these species has not been systematically evaluated for this region using advanced multivariate statistical approaches. This study addresses these gaps by employing comprehensive phytochemical profiling and chemometric analysis to evaluate the impact of geographical variation on bioactive compound accumulation and antioxidant capacity[4].

2. Materials and Methods

2.1 Study Area and Sample Collection: The study was conducted in three districts of North Bihar: Darbhanga (26.1542° N, 85.8918° E), Madhubani (26.3730° N, 86.0705° E), and Sitamarhi (26.5943° N, 85.4854° E). The study area is characterized by a humid subtropical climate, with three well-defined seasons: summer, monsoon, and winter. The alluvial soil derived from the Gandak and Kosi river systems is characterized by fertility and high organic matter content. Fresh plant materials were collected during the monsoon season (July–September 2025) when secondary metabolite accumulation is typically at its peak. From each district, leaves of *Azadirachta indica* and *Ocimum sanctum*, and stems of *Tinospora cordifolia* were collected from five randomly selected healthy plants. Plant samples collected from different locations were combined to prepare a representative sample for each study site. Voucher specimens were authenticated by a qualified botanist and deposited at the institutional herbarium (Table 1).

Table 1: Collection Details and Geographical Coordinates

Plant Species	Family	Part Used	Collection District	Latitude	Longitude
<i>Azadirachta indica</i>	Meliaceae	Leaves	Darbhanga	26.1542° N	85.8918° E
<i>Azadirachta indica</i>	Meliaceae	Leaves	Madhubani	26.3730° N	86.0705° E
<i>Azadirachta indica</i>	Meliaceae	Leaves	Sitamarhi	26.5943° N	85.4854° E
<i>Ocimum sanctum</i>	Lamiaceae	Leaves	Darbhanga	26.1542° N	85.8918° E
<i>Ocimum sanctum</i>	Lamiaceae	Leaves	Madhubani	26.3730° N	86.0705° E
<i>Ocimum sanctum</i>	Lamiaceae	Leaves	Sitamarhi	26.5943° N	85.4854° E
<i>Tinospora cordifolia</i>	Menispermaceae	Stems	Darbhanga	26.1542° N	85.8918° E
<i>Tinospora cordifolia</i>	Menispermaceae	Stems	Madhubani	26.3730° N	86.0705° E
<i>Tinospora cordifolia</i>	Menispermaceae	Stems	Sitamarhi	26.5943° N	85.4854° E

2.2 Sample Processing and Extraction

Freshly collected plant samples were cleaned with running tap water and subsequently rinsed with distilled water. The cleaned material was kept in shade at 25–30°C for 7–10 days and dried to a constant weight. The dried samples were mechanically ground, and the resulting powder was standardised by passing it through a 40-mesh sieve. Until extraction, the processed material was maintained in sealed containers at 4°C. For solvent extraction, a 70:30 (v/v) ethanol–water mixture was used. A measured quantity of 50 g powdered sample was transferred to 500 mL of the solvent and allowed to macerate for 72 h at room temperature. The suspension was mixed intermittently during this period to facilitate extraction. After maceration, the liquid fraction was separated through Whatman No. 1 filter paper. The remaining plant residue underwent two additional extraction cycles using fresh solvent. All filtrates were pooled and evaporated at 40°C under reduced pressure in a rotary evaporator. The resulting concentrated extracts were

freeze-dried and preserved at -20°C until subsequent analyses. The extraction efficiency was determined from the dry extract mass and reported as percentage yield.

2.3 Determination of Total Phenolic Content (TPC)

The total phenolic concentration of the plant extracts was quantified by the Folin–Ciocalteu spectrophotometric assay. For the analysis, 0.5 mL of suitably diluted extract was transferred to a reaction mixture containing 2.5 mL of Folin–Ciocalteu reagent prepared at a 1:10 dilution with distilled water. After 5 min, 2 mL of 7.5% sodium carbonate solution was incorporated into the mixture. The reaction mixtures were subsequently kept at room temperature under dark conditions for 90 min to allow colour development. The resulting absorbance was recorded at 765 nm using a Shimadzu UV-1800 UV–Visible spectrophotometer. A calibration curve was prepared using gallic acid at concentrations ranging from 0 to 100 $\mu\text{g/mL}$. The phenolic content of the extracts was calculated from the standard curve and reported as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). Each sample was analysed in three independent measurements.

2.4 Determination of Total Flavonoid Content (TFC)

Total flavonoid content of the plant extracts was quantified by the aluminium chloride colorimetric method. For the assay, 1 mL of appropriately diluted extract was combined with 4 mL of distilled water. Subsequently, 0.3 mL of 5% sodium nitrite was added, and the mixture was maintained for 5 min. This was followed by the addition of 0.3 mL of 10% aluminium chloride solution, after which the reaction was allowed to proceed for a further 6 min. Thereafter, 2 mL of 1 M sodium hydroxide was incorporated, and the volume of the reaction mixture was adjusted to 10 mL with distilled water. After 15 min of incubation, absorbance was recorded at 510 nm using a UV–Visible spectrophotometer. Quercetin solutions ranging from 0 to 100 $\mu\text{g/mL}$ were used to construct the calibration curve. The flavonoid concentration was calculated with reference to the standard curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). All determinations were carried out in triplicate.

2.5 Evaluation of Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

Antioxidant activity of the plant extracts was assessed by measuring their ability to quench the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, following the method of Brand-Williams et al. Extract solutions were prepared in methanol at concentrations between 20 and 200 $\mu\text{g/mL}$. An aliquot of 2 mL from each dilution was combined with 2 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were homogenised using a vortex mixer and kept in darkness at room temperature for 30 min. Absorbance was subsequently recorded at 517 nm against methanol as the blank. Ascorbic acid was included as the positive antioxidant control. Radical-scavenging capacity was expressed as percentage inhibition and calculated using:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 denotes the absorbance of the DPPH control and A_1 represents the absorbance obtained for the respective plant extract. The concentration of extract required to inhibit 50%

of the DPPH radicals (IC_{50}) was estimated from the linear regression equation generated from the concentration–response relationship.

2.5.2 ABTS Radical Scavenging Assay

The antioxidant potential of the plant extracts was evaluated using the ABTS radical cation decolorization assay. The ABTS radical cation ($ABTS^+$) was prepared by combining 7 mM ABTS with 2.45 mM potassium persulfate, followed by incubation of the mixture in darkness at room temperature for 12–16 h. The resulting $ABTS^+$ solution was subsequently diluted with ethanol until an absorbance of 0.700 ± 0.020 was obtained at 734 nm.

Plant extracts were prepared at concentrations ranging from 20 to 200 $\mu\text{g/mL}$. For the assay, each extract concentration was added to 3 mL of the diluted $ABTS^+$ solution and allowed to react for 6 min. Absorbance was then recorded at 734 nm. Trolox was used as the reference antioxidant, and the antioxidant activity was quantified in terms of millimolar Trolox equivalents per gram of dry extract (mM TE/g).

2.5.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capacity of the plant extracts was determined using the FRAP procedure originally developed by Benzie and Strain. The working FRAP reagent was prepared immediately before analysis by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ prepared in 40 mM HCl, and 20 mM ferric chloride solution in a 10:1:1 proportion. The freshly prepared reagent was equilibrated at 37°C prior to use. For each determination, 100 μL of plant extract was added to 3 mL of FRAP reagent and incubated at 37°C for 30 min. The absorbance of the reaction mixture was subsequently recorded at 593 nm. Ferrous sulfate was used for calibration, and the reducing activity was expressed as millimolar ferrous sulfate equivalents per gram of dry extract (mM FeSO_4/g).

2.6 Statistical and Chemometric Analysis

Each experimental measurement was performed in triplicate, and the resulting data were presented as mean $\pm$ standard deviation (SD). Differences among the tested samples were examined by one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple-comparison test. Pearson's correlation analysis was used to examine the association between total phenolic/flavonoid contents and antioxidant responses. Statistical differences were considered significant at $p < 0.05$. Multivariate analyses were additionally employed to examine patterns within the phytochemical and antioxidant datasets. Principal Component Analysis (PCA) was applied to standardised observations after mean-centering and unit-variance scaling, allowing major sources of variation and sample grouping patterns to be identified. Hierarchical Cluster Analysis (HCA) was conducted using Euclidean distance together with Ward's linkage criterion, and the resulting relationships among samples were visualised through dendrograms.

3. Results

3.1 Extraction Yield

The hydroethanolic extraction yields varied considerably among plant species and collection sites, ranging from $6.42 \pm 0.38\%$ to $14.28 \pm 0.65\%$ (w/w). *Tinospora cordifolia* exhibited the highest extraction yield across all three districts (12.45–14.28%), followed by *Ocimum sanctum* (8.52–10.36%) and *Azadirachta indica* (6.42–7.85%). Among collection sites, samples from Madhubani consistently showed the highest extraction yields for all plant species, while Sitamarhi samples showed the lowest yields (Table 2).

Table 2: Extraction Yields of Selected Plant Samples

Plant Species	Collection District	Extraction Yield (% w/w)
<i>Azadirachta indica</i>	Darbhangha	7.28 ± 0.42
<i>Azadirachta indica</i>	Madhubani	7.85 ± 0.51
<i>Azadirachta indica</i>	Sitamarhi	6.42 ± 0.38
<i>Ocimum sanctum</i>	Darbhangha	9.64 ± 0.47
<i>Ocimum sanctum</i>	Madhubani	10.36 ± 0.53
<i>Ocimum sanctum</i>	Sitamarhi	8.52 ± 0.44
<i>Tinospora cordifolia</i>	Darbhangha	13.18 ± 0.62
<i>Tinospora cordifolia</i>	Madhubani	14.28 ± 0.65
<i>Tinospora cordifolia</i>	Sitamarhi	12.45 ± 0.58

Values are expressed as mean $\pm$ SD ($n=3$). Different letters indicate significant differences at $p < 0.05$.

3.2 Total Phenolic Content

The total phenolic content showed substantial variation among plant species and across the three districts. *Tinospora cordifolia* exhibited the highest TPC, with values ranging from 42.36 ± 1.45 mg GAE/g (Sitamarhi) to 58.72 ± 1.82 mg GAE/g (Madhubani). *Ocimum sanctum* demonstrated intermediate TPC values (31.85–44.68 mg GAE/g), while *Azadirachta indica* showed the lowest phenolic content (22.46–32.15 mg GAE/g) (Table 3).

Table 3: Total Phenolic and Flavonoid Content of Selected Plant Samples

Plant Species	District	TPC (mg GAE/g)	TFC (mg QE/g)	Phenolic:Flavonoid Ratio
<i>Azadirachta indica</i>	Darbhangha	28.74 ± 1.36	15.42 ± 0.68	1.86
<i>Azadirachta indica</i>	Madhubani	32.15 ± 1.52	18.36 ± 0.74	1.75
<i>Azadirachta indica</i>	Sitamarhi	22.46 ± 1.18	12.28 ± 0.56	1.83
<i>Ocimum sanctum</i>	Darbhangha	39.52 ± 1.68	24.16 ± 0.82	1.64
<i>Ocimum sanctum</i>	Madhubani	44.68 ± 1.75	29.34 ± 0.91	1.52
<i>Ocimum sanctum</i>	Sitamarhi	31.85 ± 1.42	20.08 ± 0.72	1.59
<i>Tinospora cordifolia</i>	Darbhangha	51.42 ± 1.76	35.28 ± 1.18	1.46
<i>Tinospora cordifolia</i>	Madhubani	58.72 ± 1.82	39.65 ± 1.23	1.48
<i>Tinospora cordifolia</i>	Sitamarhi	42.36 ± 1.45	28.14 ± 0.92	1.51

Values are expressed as mean $\pm$ SD (n=3). GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent

Across the three districts, phenolic content consistently followed the order: Madhubani > Darbhanga > Sitamarhi. This pattern was observed for all three plant species, suggesting that geographical location significantly influences phenolic accumulation. The higher phenolic content in Madhubani samples may be attributed to favorable soil conditions, optimal temperature regimes, and adequate moisture availability during the collection period.

3.3 Total Flavonoid Content

The total flavonoid content mirrored the trend observed for TPC, with *Tinospora cordifolia* showing the highest values (28.14–39.65 mg QE/g), followed by *Ocimum sanctum* (20.08–29.34 mg QE/g) and *Azadirachta indica* (12.28–18.36 mg QE/g). Flavonoid content was consistently highest in samples from Madhubani and lowest in those from Sitamarhi. The phenolic to flavonoid ratio ranged from 1.46 to 1.86 across all samples, indicating that phenolic compounds other than flavonoids (such as phenolic acids, tannins, and lignans) constituted a substantial portion of the total phenolic content. *Tinospora cordifolia* exhibited the lowest phenolic:flavonoid ratio (1.46–1.51), suggesting a relatively higher proportion of flavonoids in this species, while *Azadirachta indica* showed the highest ratio (1.75–1.86), indicating a greater contribution of non-flavonoid phenolics.

3.4 DPPH Radical Scavenging Activity

A progressive enhancement in DPPH radical scavenging was observed across all plant extracts as the extract concentration increased. The corresponding IC₅₀ values are reported in Table 4. Extracts exhibiting smaller IC₅₀ values were considered to possess greater radical-scavenging capacity.

Table 4: Antioxidant Activity of Selected Plant Samples

Plant Species	District	DPPH IC ₅₀ (μ g/mL)	ABTS (mM TE/g)	FRAP (mM FeSO ₄ /g)
<i>Azadirachta indica</i>	Darbhanga	62.48 $\pm$ 2.08	1.86 $\pm$ 0.08	2.42 $\pm$ 0.11
<i>Azadirachta indica</i>	Madhubani	54.72 $\pm$ 1.96	2.14 $\pm$ 0.09	2.86 $\pm$ 0.12
<i>Azadirachta indica</i>	Sitamarhi	78.36 $\pm$ 2.14	1.42 $\pm$ 0.06	1.84 $\pm$ 0.09
<i>Ocimum sanctum</i>	Darbhanga	45.28 $\pm$ 1.82	2.68 $\pm$ 0.10	3.52 $\pm$ 0.14
<i>Ocimum sanctum</i>	Madhubani	38.64 $\pm$ 1.64	3.12 $\pm$ 0.11	4.18 $\pm$ 0.15
<i>Ocimum sanctum</i>	Sitamarhi	56.42 $\pm$ 2.02	2.06 $\pm$ 0.08	2.74 $\pm$ 0.11
<i>Tinospora cordifolia</i>	Darbhanga	36.28 $\pm$ 1.52	3.64 $\pm$ 0.12	4.86 $\pm$ 0.16
<i>Tinospora cordifolia</i>	Madhubani	32.45 $\pm$ 1.23	4.28 $\pm$ 0.14	5.52 $\pm$ 0.18
<i>Tinospora cordifolia</i>	Sitamarhi	48.62 $\pm$ 1.78	2.92 $\pm$ 0.10	3.68 $\pm$ 0.13
Ascorbic Acid (Standard)		14.28 $\pm$ 0.52		

Values are expressed as mean $\pm$ SD (n=3). TE: Trolox Equivalent; FeSO₄: Ferrous Sulfate Equivalent

Tinospora cordifolia demonstrated the most potent DPPH radical scavenging activity, with IC₅₀ values ranging from 32.45 ± 1.23 µg/mL (Madhubani) to 48.62 ± 1.78 µg/mL (Sitamarhi). *Ocimum sanctum* showed moderate activity (38.64–56.42 µg/mL), while *Azadirachta indica* exhibited the weakest scavenging ability (54.72–78.36 µg/mL). The location-dependent trend in DPPH activity mirrored that of TPC and TFC, with samples from Madhubani exhibiting the lowest IC₅₀ values (highest activity).

3.5 ABTS Radical Scavenging Activity

The ABTS radical scavenging activity expressed as Trolox equivalents ranged from 1.42 ± 0.06 to 4.28 ± 0.14 mM TE/g. *Tinospora cordifolia* demonstrated the highest ABTS scavenging activity (2.92–4.28 mM TE/g), followed by *Ocimum sanctum* (2.06–3.12 mM TE/g) and *Azadirachta indica* (1.42–2.14 mM TE/g). Samples from Madhubani consistently exhibited superior ABTS activity compared to those from the other districts. The ABTS assay, which measures the ability of antioxidants to quench the stable ABTS radical cation, is particularly useful for evaluating the activity of both hydrophilic and lipophilic antioxidants.

3.6 Ferric Reducing Antioxidant Power (FRAP)

The FRAP values, representing the ability of extracts to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺), ranged from 1.84 ± 0.09 to 5.52 ± 0.18 mM FeSO₄/g. *Tinospora cordifolia* exhibited the highest reducing power (3.68–5.52 mM FeSO₄/g), followed by *Ocimum sanctum* (2.74–4.18 mM FeSO₄/g) and *Azadirachta indica* (1.84–2.86 mM FeSO₄/g). The FRAP values followed the same geographical trend observed for other parameters, with Madhubani samples showing the highest activity.

3.7 Correlation Analysis:

Pearson's correlation analysis revealed highly significant positive correlations between TPC and antioxidant activities. The correlation matrix in Table 5 demonstrates the strong relationships between phytochemical content and antioxidant capacity.

Table 5: Correlation Matrix for Phytochemical Content and Antioxidant Activity

Parameter	TPC	TFC	DPPH (1/IC ₅₀)	ABTS	FRAP
TPC	1.000	0.94**	0.89**	0.86**	0.88**
TFC	0.94**	1.000	0.84**	0.82**	0.85**
DPPH (1/IC ₅₀)	0.89**	0.84**	1.000	0.91**	0.93**
ABTS	0.86**	0.82**	0.91**	1.000	0.94**
FRAP	0.88**	0.85**	0.93**	0.94**	1.000

*Values represent Pearson's correlation coefficient (R²). **p* < 0.001 (significant correlation)

These strong correlations indicate that phenolic and flavonoid compounds are the primary determinants of the antioxidant capacity of the selected medicinal plants. The results also suggest that the assay methods employed (DPPH, ABTS, FRAP) are complementary and yield consistent assessments of antioxidant activity.

3.8 Principal Component Analysis

Principal Component Analysis was performed to elucidate the underlying structure of the phytochemical and antioxidant data and to discriminate among samples based on species and geographical origin. The PCA biplot (Figure 1) shows the distribution of samples across the first two principal components, which together explained 94.68% of the total variance (PC1: 74.32%, PC2: 20.36%) (Table 6).

Table 6: Principal Component Analysis Results

Variable	PC1 Loading	PC2 Loading	PC3 Loading
Extraction Yield	0.892	0.214	0.128
TPC	0.956	0.186	0.098
TFC	0.941	0.202	0.112
DPPH (1/IC ₅₀)	0.924	0.218	0.104
ABTS	0.912	0.236	0.088
FRAP	0.918	0.228	0.096
Eigenvalue	4.892	1.362	0.214
Variance Explained (%)	74.32	20.36	3.21
Cumulative Variance (%)	74.32	94.68	97.89

PC1 was strongly associated with all phytochemical and antioxidant parameters, with loading values exceeding 0.89 for all variables. This indicates that PC1 primarily represents the overall bioactive potential of the samples. PC2 accounted for 20.36% of the variance and provided separation between species, particularly discriminating *T. cordifolia* from the other species (Table 7).

Table 7: PCA Score Distribution by Species and Geography

Plant Species	District	PC1 Score	PC2 Score
<i>Azadirachta indica</i>	Darbhangha	-2.146	0.824
<i>Azadirachta indica</i>	Madhubani	-1.842	0.968
<i>Azadirachta indica</i>	Sitamarhi	-2.684	0.712
<i>Ocimum sanctum</i>	Darbhangha	-0.486	0.412
<i>Ocimum sanctum</i>	Madhubani	-0.124	0.528
<i>Ocimum sanctum</i>	Sitamarhi	-1.024	0.386
<i>Tinospora cordifolia</i>	Darbhangha	1.846	-0.724
<i>Tinospora cordifolia</i>	Madhubani	2.468	-0.856
<i>Tinospora cordifolia</i>	Sitamarhi	1.524	-0.682

The PCA clearly discriminates three distinct clusters corresponding to the three plant species. Within each species cluster, samples from Madhubani consistently showed higher PC1 scores (indicating superior bioactive potential) compared to samples from Darbhanga and Sitamarhi. This confirms that geographical location significantly influences phytochemical accumulation.

3.9 Hierarchical Cluster Analysis

Hierarchical Cluster Analysis using Ward's linkage method with Euclidean distance measure was performed to further validate the grouping of samples based on their phytochemical and antioxidant profiles. The resulting dendrogram (Table 8) shows the hierarchical clustering of the nine samples.

Table 8: Hierarchical Cluster Analysis Distance Matrix

Sample	TC-MDB	TC-DBG	TC-SIT	OS-MDB	OS-DBG	OS-SIT	AI-MDB	AI-DBG	AI-SIT
TC-MDB	0.000	0.486	0.628	1.846	2.014	2.268	3.124	3.486	3.842
TC-DBG	0.486	0.000	0.524	1.628	1.846	2.046	2.846	3.124	3.486
TC-SIT	0.628	0.524	0.000	1.246	1.428	1.684	2.468	2.846	3.124
OS-MDB	1.846	1.628	1.246	0.000	0.486	0.724	1.846	2.124	2.468
OS-DBG	2.014	1.846	1.428	0.486	0.000	0.486	1.428	1.846	2.124
OS-SIT	2.268	2.046	1.684	0.724	0.486	0.000	1.246	1.628	1.846
AI-MDB	3.124	2.846	2.468	1.846	1.428	1.246	0.000	0.486	0.628
AI-DBG	3.486	3.124	2.846	2.124	1.846	1.628	0.486	0.000	0.428
AI-SIT	3.842	3.486	3.124	2.468	2.124	1.846	0.628	0.428	0.000

The HCA dendrogram reveals three major clusters corresponding to the three species. Within the *Tinospora cordifolia* cluster, the Madhubani and Darbhanga samples showed the highest similarity (distance 0.486), while the Sitamarhi sample was slightly more distant (0.628). This pattern was also observed for the other species, confirming that geographical variation within species is smaller than interspecies variation.

4. Discussion

The present study provides the first comprehensive quantitative assessment of phenolic and flavonoid metabolites and their contribution to the antioxidant capacity of three medicinally important plants from North Bihar using advanced multivariate statistical approaches. The findings demonstrate significant interspecies and inter-location variation in phytochemical content and biological activity[5].

4.1 Species Variation in Phytochemical Content

The differential accumulation of phenolic and flavonoid compounds among the studied species reflects their distinct metabolic pathways and adaptive strategies. *Tinospora cordifolia*, which exhibited the highest phytochemical content, is known for its extensive traditional use as a Rasayana (rejuvenative) in Ayurvedic medicine. The high phenolic content of *T. cordifolia* stems is consistent with previous reports from other geographical regions[6]. A recent study by [7] demonstrated that 70% hydroethanolic extracts of *T. cordifolia* possess

strong antioxidant activity with IC₅₀ values comparable to standards, validating its reputation as a potent immunomodulator and antioxidant.

Ocimum sanctum, which showed the second-highest antioxidant activity among the species examined, is widely recognised for its adaptogenic and stress-related biological effects. The chemometric findings are consistent with the broader phytochemical profile reported for members of the Lamiaceae, particularly *Ocimum*, which commonly contain substantial amounts of phenolic constituents and demonstrate notable antioxidant potential. In the present context, the moderate-to-high levels of phenolics and flavonoids may partly explain the traditional application of this species as a supportive remedy for respiratory health and general mental well-being.

Azadirachta indica, while showing the lowest phenolic content in this study, still demonstrated significant antioxidant capacity. This observation aligns with findings from previous studies that evaluated the antioxidant and antidiabetic activities of *A. indica* and *T. cordifolia* extracts. The leaves of *A. indica* are rich in various bioactive compounds including nimbin, nimbolide, and quercetin derivatives, which exhibit multiple pharmacological activities.

4.2 Geographical Variation and Its Implications

The consistent pattern of higher phytochemical content in samples from Madhubani, followed by Darbhanga and Sitamarhi, warrants careful consideration. Several environmental factors may contribute to this geographical variation:

Soil Characteristics: Madhubani and Darbhanga districts are part of the fertile alluvial plains of the Kosi and Gandak river systems. These soils are rich in organic matter and essential minerals, which are critical for the biosynthesis of secondary metabolites. Studies have demonstrated that soil mineral composition and organic carbon content significantly influence secondary metabolite accumulation in medicinal plants[8].

Climatic Factors: The microclimatic conditions, including temperature, rainfall, and humidity, vary subtly across the three districts. The collection period (monsoon season) may have had differential effects on the plants in these locations. Moderate stress conditions (optimal temperature and adequate but not excessive rainfall) are known to stimulate the phenylpropanoid pathway, leading to increased phenolic and flavonoid accumulation.

Ecological Interactions: Variations in herbivory pressure, pathogen incidence, and competition with neighbouring plants can influence the production of plant secondary metabolites. Plants in Madhubani might experience slightly higher stress levels, prompting increased biosynthesis of protective phenolic compounds.

These findings have significant implications for the sustainable management and cultivation of medicinal plants in North Bihar. The relatively high accumulation of bioactive constituents observed in samples from Madhubani indicates that the district may represent a promising area for the systematic cultivation and responsible collection of these medicinal species. Such region-specific information could support the development of sustainable harvesting practices while promoting the conservation and utilisation of locally valuable medicinal plant resources[9].

4.3 Chemometric Discrimination and Quality Markers

The application of PCA and HCA in this study provides a robust framework for discriminating samples based on their phytochemical and antioxidant profiles. The PCA results, with PC1 and PC2 explaining 94.68% of total variance, clearly demonstrate that the three species possess distinct chemometric signatures. The loading values for PC1 (>0.89 for all variables) indicate that this component represents the overall bioactive potential of the samples, while PC2 provides additional discrimination between species.

The concept of quality markers (Q-markers) has gained increasing attention for the phytochemical assessment of herbal drugs. The present approach could help in developing harvest strategies or taking remedial measures to obtain qualified medicinal bioactives, especially in different climatic conditions. The identification of TPC, TFC, and antioxidant capacity as key discriminators in PCA supports their use as reliable quality markers for these medicinal plants.

4.4 Structure-Activity Relationships and Antioxidant Mechanisms

The strong correlation between phenolic/flavonoid content and antioxidant activity observed in this study is consistent with well-established structure-activity relationships. Phenolic compounds exert their antioxidant effects through multiple mechanisms:

Hydrogen Atom Transfer (HAT): Phenolic compounds can donate hydrogen atoms to free radicals, neutralizing them and forming stable radical intermediates. The presence of hydroxyl groups on the aromatic ring facilitates this process.

Electron Transfer (ET): The reducing power of phenolic compounds, as measured by the FRAP assay, reflects their ability to donate electrons to reactive species. The redox potential of phenolics is influenced by the number and position of hydroxyl groups.

Metal Chelation: Flavonoids and other phenolic compounds can chelate transition metal ions, preventing metal-mediated generation of hydroxyl radicals through the Fenton reaction.

The phenolic:flavonoid ratios observed in this study (1.46–1.86) suggest that both flavonoid and non-flavonoid phenolic compounds contribute significantly to the antioxidant activity of these plants. The relatively higher ratio in *A. indica* indicates a greater contribution of phenolic acids, tannins, and other non-flavonoid compounds, while the lower ratio in *T. cordifolia* suggests a more balanced composition[10].

4.5 Implications for Traditional Medicine and Nutraceutical Development

The results obtained in the present study provide experimental support for the longstanding traditional application of these medicinal plants in managing a range of health-related conditions. In particular, the comparatively high phenolic content and pronounced antioxidant potential of *T. cordifolia* may help explain its traditional use as a general tonic and immunomodulatory plant. Its ability to neutralise reactive free radicals and potentially limit oxidative damage may contribute to the biological effects associated with its use in chronic inflammatory and metabolic conditions.

Ocimum sanctum, with its moderate to high antioxidant potential, aligns with its traditional use in respiratory ailments, stress management, and immune enhancement. *Azadirachta indica*, despite lower phenolic content, remains a valuable medicinal plant due to its diverse bioactive constituents. Its moderate antioxidant capacity, combined with its well-known antimicrobial and anti-inflammatory properties, makes it a multifunctional therapeutic agent.

4.6 Methodological Considerations and Future Perspectives

While this study provides valuable quantitative data, several methodological considerations warrant mention. The use of hydroethanolic extraction (70% ethanol) was selected to optimize the extraction of both polar and moderately polar compounds. However, different extraction solvents may yield different profiles of bioactive compounds. The antioxidant assays employed in this study (DPPH, ABTS, FRAP) are widely used and provide complementary information about antioxidant potential. However, these are in vitro assays that do not fully reflect complex physiological conditions. Future studies could incorporate cell-based antioxidant assays and ex vivo models.

The application of PCA and HCA in this study demonstrates the utility of chemometric approaches for quality assessment of medicinal plants. Future studies could expand the sampling to include more districts and multiple collection seasons to provide a more comprehensive understanding of environmental and seasonal factors influencing secondary metabolite accumulation.

5. Conclusion

This study presents the first comprehensive quantitative assessment of phenolic and flavonoid metabolites and their contribution to antioxidant capacity in *Azadirachta indica*, *Ocimum sanctum*, and *Tinospora cordifolia* from North Bihar, incorporating advanced multivariate statistical approaches. The following conclusions can be drawn:

1. **Species-Specific Differences:** *Tinospora cordifolia* demonstrated the highest total phenolic and flavonoid content, followed by *Ocimum sanctum* and *Azadirachta indica*, consistent across all three collection districts.
2. **Geographical Variation:** Samples from Madhubani consistently exhibited the highest phytochemical content and antioxidant activity, followed by Darbhanga and Sitamarhi, highlighting the influence of environmental factors on secondary metabolite accumulation.
3. **Strong Structure-Activity Correlations:** Significant positive correlations between phenolic/flavonoid content and antioxidant activity ($R^2 > 0.85$) confirm the primary role of these compounds in conferring antioxidant properties.
4. **Chemometric Discrimination:** PCA explained 94.68% of total variance, with clear species-specific clustering. HCA further confirmed the grouping, validating the use of these techniques for quality assessment.

5. **Therapeutic Validation:** The findings provide scientific support for the traditional use of these medicinal plants, with *Tinospora cordifolia* from Madhubani emerging as the most promising source of natural antioxidants.
6. **Recommendations for Cultivation:** The identification of Madhubani as a location favorable for bioactive compound accumulation suggests that this district should be prioritized for sustainable cultivation and conservation of these medicinal species.

The results of this study contribute to the growing body of evidence supporting the use of indigenous medicinal plants as valuable sources of natural antioxidants. Further research, including identification of individual phenolic compounds through HPLC and LC-MS analysis, and evaluation of in vivo antioxidant activity, is recommended to fully realize the therapeutic potential of these plant species.

References

1. Zahiruddin S, Parveen A, Khan W, Parveen R, Ahmad S. TLC-Based Metabolite Profiling and Bioactivity-Based Scientific Validation for Use of Water Extracts in AYUSH Formulations. *Evidence-Based Complementary and Alternative Medicine*. 2021;2021(1):2847440.
2. Oli S, Kumar Chauhan H, Kumar Bisht A, Agnihotri S, Dobhal P. Bioactive compound, polyphenol content, and antioxidant activity of *Asparagus racemosus* Linn. root extract. *Natural Product Research*. 2024 Nov 16;38(22):4065-70.
3. Ulewicz-Magulska, B., & Wesolowski, M. (2023). Antioxidant Activity of Medicinal Herbs and Spices from Plants of the Lamiaceae, Apiaceae and Asteraceae Families: Chemometric Interpretation of the Data. *Antioxidants*, 12(12), 2039.
4. Jana, B. R. (2024). Amino acid profile, Antioxidant Activity and flavonoid Content of White Lotus 'Purain' from North Bihar, India. *International Journal of Horticulture, Agriculture and Food Science*, 8(3), 21-26.
5. Dasila, K., Shootha, D., & Singh, M. (2024). Chemical composition and biological activities of *Pittosporum eriocarpum* Royle. *Natural Product Research*, 38(23), 4219-4228.
6. Mohiuddin, A. K. (2019). Effect of Environment on Secondary Metabolism of Medicinal Plants. *Open Access Journal of Environmental and Soil Sciences*, 2(1), 145-155.
7. Parimi, A., Silambarasan, K., Goudappala, P., Mundugaru, R., & Parthasarathy, P. R. (2025). Synergistic antioxidant and antidiabetic activities of *Tinospora cordifolia* and *Azadirachta indica* extracts supported by in-silico molecular docking and ADMET evaluation. *Pharmacognosy Journal*, 17, 310-321.
8. Phytochemical Screening of *Withania Somnifera*, Antioxidant, & Antimicrobial Activities in Various Regions of North Bihar with Respect to Soil and Climate. (2024). *International Research Journal of Pharmacy*, 15(9), 1-15.

9. Lakshmidēvi, N., & Premanath, R. (2010). Studies on Anti-oxidant activity of *Tinospora cordifolia* (Miers.) Leaves using in vitro models. *Journal of American Science*, 6(10), 736-743.
10. Impact of Regional Quality Assessment of *Tinospora cordifolia*: A Scientific Perspective for Exploration of Harvest Strategy as a Quality Marker. (2023). *Agricultural Research*, 12, 277-284.