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# COMPARATIVE EVALUATION OF SOLVENT POLARITY ON THE EXTRACTION EFFICIENCY, PHYTOCHEMICAL RECOVERY AND ANTIOXIDANT CAPACITY OF *Ocimum sanctum* AND *Azadirachta indica*

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**ABSTRACT**

Solvent polarity plays a critical role in the extraction efficiency of bioactive phytochemicals from medicinal plants. The present study aimed to comparatively evaluate the influence of aqueous and ethanolic extraction systems on the recovery of antioxidant phytoconstituents from *Ocimum sanctum* and *Azadirachta indica*. Dried leaf powders of both plant species were subjected to aqueous maceration and ethanolic Soxhlet extraction. Quantitative phytochemical analysis was performed to determine Total Phenolic Content (TPC) using the Folin–Ciocalteu method and Total Flavonoid Content (TFC) using the Aluminum Chloride colorimetric assay. Antioxidant activity was evaluated by DPPH and ABTS radical scavenging assays. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test, with significance considered at $p < 0.05$. Ethanolic extracts exhibited significantly higher phytochemical recovery and antioxidant activity compared to aqueous extracts. The ethanolic extract of *Ocimum sanctum* showed the highest TPC (125.4 ± 3.2 mg GAE/g) and TFC (58.6 ± 2.1 mg QE/g). In antioxidant assays, O. sanctum ethanolic extract demonstrated the strongest activity with DPPH IC50 and ABTS IC50 values of 42.5 ± 1.5 µg/mL and 38.2 ± 1.2 µg/mL, respectively, approaching the activity of standard Ascorbic Acid. A strong positive correlation ($r = 0.96$, $p < 0.01$) was observed between phenolic content and antioxidant potential. The findings indicate that 95% ethanol is more effective than distilled water for extracting antioxidant-rich phytochemicals from *Ocimum sanctum* and *Azadirachta indica*. Ethanolic extraction significantly enhanced phenolic and flavonoid recovery, resulting in superior antioxidant activity. These results support the application of ethanolic extraction in the development of herbal nutraceuticals and phytopharmaceutical formulations.

***Keywords*** **:** *Ocimum sanctum*, *Azadirachta indica*, phytoconstituents, antioxidant.

## Introduction

Plants function as highly sophisticated biosynthetic laboratories capable of synthesizing a diverse array of secondary metabolites, including alkaloids, glycosides, terpenes, phenolics, and flavonoids. Unlike primary metabolites, these phytochemicals are not strictly essential for the plant's immediate survival, growth, or reproduction. Instead, they serve as critical evolutionary adaptations that provide defence against herbivorous predation, microbial infections, and abiotic environmental stressors.

From a pharmacological perspective, these secondary metabolites exhibit profound therapeutic utility in human physiology. A major mechanism underlying their medical efficacy is their capacity to

function as potent exogenous antioxidants. These compounds actively neutralize Reactive Oxygen Species (ROS) such as superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (. OH) which induce cellular oxidative stress when generated in excess. (Hakkim *et al*., 2007; Pham-Huy *et al*., 2008). Left unchecked, chronic oxidative stress drives lipid peroxidation, nucleic acid mutations, and enzymatic degradation. These biochemical disruptions are heavily implicated in the pathogenesis of numerous chronic diseases, including various forms of cancer, cardiovascular disorders, and progressive neurodegenerative conditions like Alzheimer's and Parkinson's disease(Pham-Huy *et al*., 2008; Vasincu *et al*., 2025).

The therapeutic potency of any medicinal plant preparation is fundamentally dictated by both the quantitative yield and qualitative profile of these bioactive constituents. However, isolating these target compounds from the intricate intracellular matrix of plant tissues represents a complex biochemical challenge. Phytochemical extraction operates under the thermodynamic principle of like dissolves like, meaning the chemical polarity of the extraction solvent must match that of the target secondary metabolites to maximize solubility and mass transfer(Do *et al*., 2014; Tripathi *et al*., 2025; Wylie & Merrell, 2022).

Historically, traditional medical practices have relied heavily on aqueous preparations, such as decoctions and infusions, to extract water-soluble plant components. In contrast, modern pharmaceutical and nutraceutical industries frequently favor organic solvents such as ethanol, methanol, or ethyl acetate or hydroalcoholic mixtures. These organic solvents often yield higher extraction efficiencies, broader chemical profiles, and superior long-term stability for the isolated bioactives(Tourabi *et al*., 2025; Tripathi *et al*., 2025). Medium-polar to polar organic solvents (with polarity indices ranging between 4.1 and 5.2) are particularly effective at breaking down plant cell walls and dissolving complex polyphenolic and flavonoid fractions(Tripathi *et al*., 2025).

Within the ethnomedicinal framework of the North-east region of India, *Ocimum sanctum* (Holy Basil or Tulsi) and *Azadirachta indica* (Neem) are two of the most widely utilized therapeutic plants.

- *Ocimum sanctum* (Tulsi): Renowned for its adaptogenic properties, its pharmacological activity is largely driven by a dense volatile and non-volatile chemical profile. Key bioactive markers include ursolic acid (a pentacyclic triterpenoid), eugenol (an allylbenzene), and rosmarinic acid (a phenolic ester), which together exert robust radical-scavenging and anti-inflammatory activities(Hakkim *et al*., 2007; Yadav *et al*., 2024).
- *Azadirachta indica* (Neem): Characterized by a complex array of bitter tetranortriterpenoids and polyphenols. Its most prominent therapeutic constituents include azadirachtin (highly regarded for its antimicrobial properties), nimbin, and the flavonol quercetin, which exhibits exceptional electron-donating capacities to neutralize free radicals(Alzohairy, 2016; Sarkar *et al*., 2021; Wylie & Merrell, 2022).

Despite their extensive regional use in traditional North-east remedies, there remains a critical gap in rigorous, comparative quantitative data regarding how different extraction methodologies influence the definitive antioxidant yield of these specific local populations. Variations in regional soil chemistry, climate, and altitude drastically modulate the synthesis of secondary metabolites in both *Ocimum sanctum* and *Azadirachta indica* (Yadav *et al*., 2024).Consequently, empirical data evaluating the optimal solvent system for these regional chemotypes is severely lacking.

This research aims to bridge this regional and pharmacological gap by executing a systematic, comparative quantitative analysis of the phytochemical profiles and antioxidant capacities of *Ocimum sanctum* and *Azadirachta indica* extracted via pure aqueous versus ethanolic systems. By evaluating total phenolic content, total flavonoid content, and real-time free-radical scavenging efficiencies, this study seeks to identify the optimal solvent architecture to maximize the industrial scale-up and therapeutic translation of these botanical assets.

## Materials and Methods

**Chemicals and Reagents:** 2,2-diphenyl-1-picrylhydrazyl (DPPH) 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Gallic acid, Quercetin, Folin Ciocalteu reagent, Sodium carbonate and Aluminum chloride were of analytical grade (Sigma Aldrich, USA). Ethanol (95%) and other solvents were procured from the Department of Pharmacognosy laboratory.

**Plant Material Collection and Processing:** Fresh leaves of *Ocimum sanctum* and *Azadirachta indica* were collected from the NIPS campus, Kamrup, in Jan 2026. The leaves were washed thoroughly with deionized water to remove surface pollutants and shade dried at room temperature (25-30 degrees Celsius) for 14 days. The dried leaves were pulverized into a fine powder using a mechanical grinder and sieved (mesh

size 60) to ensure uniform particle size.

**Extraction Procedure:** Two parallel extraction methods were employed for each plant species:

**1.Ethanolic Extraction (Soxhlet Method):** 50 g of dried plant powder was placed in a porous thimble and extracted with 250 mL of 95% Ethanol in a Soxhlet apparatus for 12 hours at 60 degrees Celsius. This continuous hot extraction method ensures exhaustive extraction of thermostable compounds. Ethanol (95%) was selected due to its intermediate polarity and ability to efficiently extract both phenolic and flavonoid compounds (Tourabi *et al*., 2025).

**Aqueous Extraction (Maceration Method):** 50 g of dried plant powder was soaked in 250 mL of sterile distilled water in a conical flask. The mixture was kept on a rotary shaker (150 rpm) for 48 hours at room temperature. This mimics traditional decoction methods(Nn, 2015).

**Filtration and Yield Calculation:** Both extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator and weighed to calculate the percentage yield: Yield (%) = (Weight of dried extract / Weight of plant powder) x 100(Sasidharan *et al*., 2011).

**Quantitative Phytochemical Analysis**

**Determination of Total Phenolic Content (TPC):** TPC was estimated using the Folin Ciocalteu spectrophotometric method (Singleton *et al*., 1999).

**Protocol:** 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin Ciocalteu reagent and 2 mL of 7.5% Sodium Carbonate ($Na_2CO_3$). The mixture was incubated at 45 degrees Celsius for 15 minutes.

**Measurement:** Absorbance was measured at 765 nm.

**Standard:** A calibration curve of Gallic Acid (20–100 µg/mL) was prepared. Results were expressed as mg Gallic Acid Equivalents (GAE) per gram of dry extract.

**Determination of Total Flavonoid Content (TFC):** TFC was measured using the Aluminum Chloride colorimetric assay(Chang *et al*., 2002).

**Protocol:** 0.5 mL of extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% Aluminum Chloride ($AlCl_3$) 0.1 mL of 1M Potassium Acetate and 2.8 mL of distilled water.

**Measurement:** After 30 minutes of incubation at room temperature, absorbance was measured at 415 nm.

**Standard:** Quercetin was used as the standard. Results were expressed as mg Quercetin Equivalents (QE) per gram of dry extract.

**In Vitro Antioxidant Assays**

DPPH Radical Scavenging Assay: This assay measures the ability of the extract to donate hydrogen atoms to the stable free radical DPPH(Blois, 1958).

**Protocol:** 1 mL of 0.1 mM DPPH solution (in methanol) was added to 3 mL of extract at varying concentrations (20, 40, 60, 80, 100 µg/mL).

**Measurement:** The mixture was shaken vigorously and incubated in the dark for 30 minutes. Absorbance was read at 517 nm against a methanol blank.

**ABTS Radical Cation Decolorization Assay:** This assay assesses the scavenging activity against the ABTS radical cation (ABTS+) which is applicable for both hydrophilic and lipophilic antioxidant systems.

**Protocol:** ABTS radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate for 16 hours in the dark. The solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 1 mL of diluted ABTS solution was mixed with 10 microliters of extract.

**Measurement:** Absorbance was measured at 734 nm after 6 minutes (Re *et al*., 1999).

**Statistical Analysis**

All experiments were carried out in triplicate (n = 3), and the results were expressed as Mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among extraction groups. Student's t-test was used for pairwise comparison between ethanolic and aqueous extracts where appropriate. A value of $p < 0.05$ was considered statistically significant. Pearson correlation analysis was performed to evaluate the relationship between Total Phenolic Content (TPC) and antioxidant activity. Statistical calculations were performed using GraphPad Prism version 9.0 (GraphPad Software, USA).

## Results and Discussion

The choice of extraction solvent significantly influenced the yield of bioactive compounds and their subsequent antioxidant activity. The results demonstrate that ethanol is a superior solvent for extracting phenolics and flavonoids compared to water.

**Extraction Yield and Phytochemical Content**

The extraction yield varied between the two solvents, with water showing a higher mass yield (likely due to the extraction of sugars and soluble proteins). However, the specific yield of bioactive phenolics (TPC) and flavonoids (TFC) was

significantly higher in the ethanolic extracts. The ethanolic extracts of both plant species exhibited significantly higher Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) compared to aqueous extracts ($p < 0.05$). Similarly, antioxidant assays demonstrated significantly lower IC50 values for ethanolic extracts, indicating superior free radical scavenging activity (Do *et al*., 2014).

The comparative efficiency of 95% Ethanol and Distilled Water in recovering bioactive metabolites is illustrated in Figure 1.

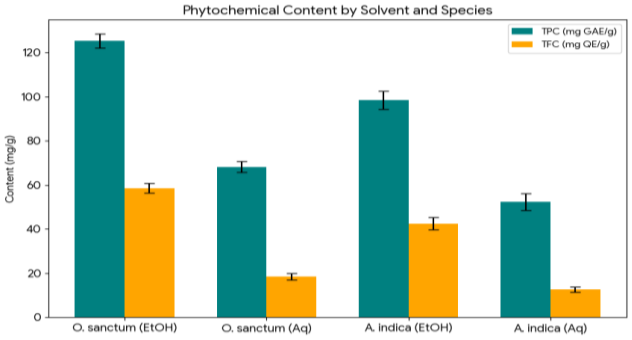


**Fig. 1:** Comparison of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in *Ocimum sanctum* and *Azadirachta indica* using different solvent systems

Total Phenolic Content (TPC): Phenolic compounds are polar but their solubility is often enhanced in organic solvents like ethanol due to the presence of hydroxyl groups.

- *Ocimum sanctum* (Ethanol): 125.4 ± 3.2 mg GAE/g
- *Ocimum sanctum* (Aqueous): 68.2 ± 2.5 mg GAE/g
- *Azadirachta indica* (Ethanol): 98.6 ± 4.1 mg GAE/g
- *Azadirachta indica* (Aqueous): 52.4 ± 3.8 mg GAE/g

Total Flavonoid Content (TFC): Flavonoids generally have low solubility in water. Consequently, the ethanolic extracts showed a 2-3fold higher concentration of flavonoids compared to the aqueous extracts.

**Table 1:** Quantitative Phytochemical Analysis

| Plant Species | Solvent | TPC (mg GAE/g) | TFC (mg GAE/g) |
|---|---|---|---|
| *Ocimum sanctum* | Ethanol | 125.4 ± 3.2 | 58.6 ± 2.1 |
| *Ocimum sanctum* | Aqueous | 68.2 ± 2.5 | 18.4 ± 1.5 |
| *Azadirachta indica* | Ethanol | 98.6 ± 4.1 | 42.5 ± 2.8 |
| *Azadirachta indica* | Aqueous | 52.4 ± 3.8 | 12.6 ±1.2 |

(Values are Mean ± SD of triplicates. GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents: Values are expressed as Mean ± SD (n = 3). Different superscript letters indicate statistically significant differences ($p < 0.05$))

**Table 2:** Raw Triplicate Data for TPC and TFC Analysis

| Sample | Rep 1 (TPC) | Rep 2 (TPC) | Rep 3 (TPC) | Mean ± SD (TPC) | Rep 1 (TFC) | Rep 2 (TFC) | Rep 3 (TFC) | Mean ± SD (TFC) |
|---|---|---|---|---|---|---|---|---|
| *O. sanctum* (Ethanol) | 122.8 | 126.7 | 126.7 | 125.4 ± 3.2 | 56.4 | 59.7 | 59.7 | 58.6 ± 2.1 |
| *O. sanctum* (Aqueous) | 65.9 | 69.5 | 69.2 | 68.2 ± 2.5 | 17.1 | 19.2 | 18.9 | 18.4 ± 1.5 |
| *A. indica* (Ethanol) | 94.8 | 99.6 | 101.4 | 98.6 ± 4.1 | 39.8 | 43.6 | 44.1 | 42.5 ± 2.8 |
| *A. indica* (Aqueous) | 48.7 | 54.9 | 53.6 | 52.4 ± 3.8 | 11.4 | 13.1 | 13.3 | 12.6 ± 1.2 |

(Values are expressed as Mean ± SD of triplicate measurements (n = 3))

**Antioxidant Activity (DPPH Assay)**

The DPPH assay revealed a dose-dependent scavenging activity for all extracts. The ethanolic extract of *Ocimum sanctum* exhibited the highest potency, with an $IC_{50}$ value of 42.5 µg/mL, which is comparable to the standard Ascorbic Acid ($IC_{50}$ = 35.2 µg/mL). The aqueous extracts showed significantly weaker activity (higher $IC_{50}$) indicating that many potent antioxidants in these plants are lipophilic or semi polar and are not effectively extracted by water alone.

The DPPH radical scavenging assay demonstrated concentration-dependent antioxidant activity for all extracts. Ethanolic extracts exhibited significantly higher percentage inhibition compared to aqueous extracts at all tested concentrations ($p < 0.05$). *Ocimum sanctum* ethanolic extract showed the strongest scavenging activity among plant extracts, approaching the activity of the standard Ascorbic Acid.

DPPH scavenging activity was calculated using:

$$\%\ Inhibition = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

- $A_0$= absorbance of control
- $A_1$= absorbance of sample extract

**Table 3:** DPPH Radical Scavenging Activity (% Inhibition)

| Concentration (µg/mL) | *O. sanctum* (Ethanol) | *O. sanctum* (Aqueous) | *A. indica* (Ethanol) | *A. indica* (Aqueous) | Ascorbic Acid |
|---|---|---|---|---|---|
| 20 | 24.6 ± 1.2 | 12.4 ± 0.8 | 18.5 ± 1.0 | 9.6 ± 0.7 | 31.8 ± 1.1 |
| 40 | 41.2 ± 1.5 | 24.8 ± 1.2 | 33.4 ± 1.3 | 18.7 ± 0.9 | 52.6 ± 1.4 |
| 60 | 58.7 ± 1.8 | 37.5 ± 1.5 | 49.2 ± 1.7 | 29.8 ± 1.2 | 68.9 ± 1.7 |
| 80 | 74.3 ± 2.1 | 51.4 ± 1.8 | 63.7 ± 2.0 | 41.5 ± 1.5 | 84.2 ± 2.0 |
| 100 | 89.5 ± 2.4 | 64.8 ± 2.2 | 78.4 ± 2.3 | 53.2 ± 1.9 | 95.6 ± 2.3 |

(Values are expressed as Mean ± SD, n = 3)

The radical scavenging potencies ($IC_{50}$) of the plant extracts against DPPH and ABTS radicals are visually summarized in Figure 2.

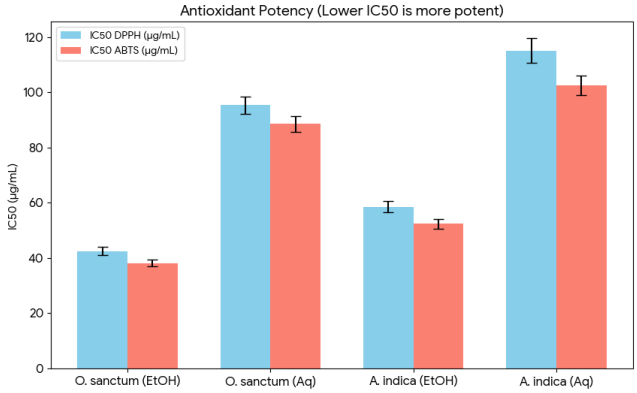


**Fig. 2:** Lower $IC_{50}$ values represent higher antioxidant potency. Statistical significance was evaluated using one-way ANOVA followed by Tukey's test ($p < 0.05$)

**Table 4:** Antioxidant Activity ($IC_{50}$ Values)

| Extract | $IC_{50}$ (DPPH) (µg/mL) | $IC_{50}$ (ABTS) (µg/mL) |
|---|---|---|
| *O. sanctum* (Ethanol) | 42.5 ± 1.5 | 38.2 ± 1.2 |
| *O. sanctum* (Aqueous) | 95.4 ± 3.2 | 88.6 ± 2.8 |
| *A. indica* (Ethanol) | 58.6 ± 2.1 | 52.4 ± 1.8 |
| *A. indica* (Aqueous) | 115.2 ± 4.5 | 102.5 ± 3.6 |
| *Ascorbic Acid* (Std) | 35.2 ± 1.2 | 30.5 ± 1.0 |

(Values are expressed as Mean ± SD (n = 3). Lower $IC_{50}$ values indicate stronger antioxidant activity. Differences between ethanolic and aqueous extracts were statistically significant ($p < 0.05$))

**Table 5:** Raw Triplicate Data for Antioxidant Activity (IC50 Values)

| Extract | Replicate 1 (DPPH) | Replicate 2 (DPPH) | Replicate 3 (DPPH) | Mean ± SD (DPPH) | Replicate 1 (ABTS) | Replicate 2 (ABTS) | Replicate 3 (ABTS) | Mean ± SD (ABTS) |
|---|---|---|---|---|---|---|---|---|
| *O. sanctum* (Ethanol) | 40.9 | 42.8 | 43.8 | 42.5 ± 1.5 | 36.9 | 38.4 | 39.3 | 38.2 ± 1.2 |
| *O. sanctum* (Aqueous) | 91.8 | 96.7 | 97.7 | 95.4 ± 3.2 | 85.4 | 89.7 | 90.7 | 88.6 ± 2.8 |
| *A. indica* (Ethanol) | 56.1 | 58.9 | 60.8 | 58.6 ± 2.1 | 50.3 | 52.7 | 54.2 | 52.4 ± 1.8 |
| *A. indica* (Aqueous) | 110.4 | 116.7 | 118.5 | 115.2 ± 4.5 | 98.7 | 103.8 | 105.0 | 102.5 ± 3.6 |
| Ascorbic Acid (Std) | 33.8 | 35.4 | 36.4 | 35.2 ± 1.2 | 29.2 | 30.6 | 31.7 | 30.5 ± 1.0 |

(Values are expressed as Mean ± SD of triplicate measurements (n = 3). Lower IC50 values indicate higher antioxidant potency)

All experiments were performed in triplicate and data were expressed as Mean ± Standard Deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant

## Correlation Analysis

A strong positive linear correlation was observed between Total Phenolic Content (TPC) and DPPH radical scavenging activity, with Pearson's correlation coefficient of $r = 0.96 (p < 0.01)$. Linear regression analysis demonstrated that increased phenolic

concentration was associated with enhanced antioxidant activity

A linear regression analysis (Figure 3) further confirms the strong positive correlation between phenolic concentration and free radical scavenging activity.

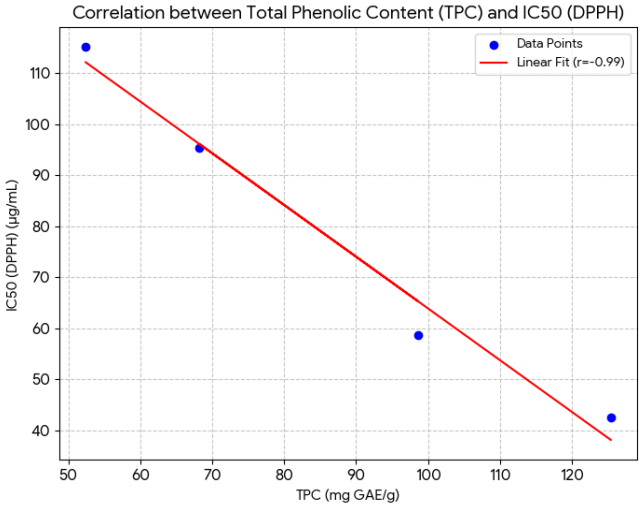


**Fig. 3:** Linear correlation between Total Phenolic Content (TPC) and DPPH radical scavenging activity (r = 0.96)

## Conclusion

The present study demonstrates that solvent polarity exerts a significant influence on the extraction efficiency, phytochemical recovery, and antioxidant performance of medicinal plant extracts derived from *Ocimum sanctum* and *Azadirachta indica*. Under the experimental conditions employed, 95% ethanolic extraction consistently yielded higher concentrations of total phenolics and flavonoids compared with aqueous extraction, which was further reflected in enhanced DPPH and ABTS radical scavenging activities. Among the investigated samples, the ethanolic extract of *Ocimum sanctum* exhibited the most pronounced antioxidant potential, suggesting a higher abundance of bioactive redox-active phytoconstituents.

The strong positive correlation observed between Total Phenolic Content and antioxidant activity supports the critical contribution of phenolic compounds to the free-radical scavenging capacity of these plant extracts. These findings are consistent with the growing body of evidence indicating that hydroalcoholic and moderately polar solvent systems improve the recovery of antioxidant phytochemicals from medicinal plant matrices.

Collectively, the study provides experimental evidence supporting the selection of ethanolic extraction systems for the preparation of antioxidant-rich herbal formulations and nutraceutical applications. Nevertheless, the present investigation remains limited to spectrophotometric phytochemical estimation and in vitro antioxidant evaluation. Further studies involving chromatographic characterization, compound isolation, bioavailability assessment, and in vivo pharmacological validation are warranted to establish the therapeutic relevance and translational potential of these extracts for pharmaceutical and functional food applications.

**Conflict of Interest:**
The authors declare no conflict of interest.

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