

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF SELECTED MEDICINAL PLANTS

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Abstract

Medicinal plants represent a significant source of bioactive secondary metabolites with potential therapeutic applications, particularly as natural antioxidants. The present study investigates the phytochemical composition and antioxidant activity of selected medicinal plant species using standardized extraction and analytical methods. Dried plant materials were subjected to solvent extraction using methanol and ethanol to obtain crude extracts rich in phytoconstituents. Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) was performed using spectrophotometric techniques, while antioxidant capacity was evaluated through DPPH radical scavenging, ABTS decolorization, and ferric reducing antioxidant power (FRAP) assays. The results demonstrated notable variability among plant species, with higher phenolic and flavonoid contents generally associated with stronger antioxidant activity. However, the relationship was not uniformly consistent, indicating the contribution of other bioactive compounds and possible synergistic effects. While the findings support the potential of medicinal plants as sources of natural antioxidants, the reliance on crude extracts and *in vitro* assays limits direct pharmacological interpretation. Further studies involving compound isolation, structural characterization, and *in vivo* validation are required to substantiate their therapeutic efficacy.

Introduction

Medicinal plants have long served as a foundational component of traditional healthcare systems and continue to be a prolific source of bioactive compounds with therapeutic potential. The pharmacological relevance of these

plants is primarily attributed to their diverse array of secondary metabolites, including phenolics, flavonoids, alkaloids, tannins, and terpenoids, which exhibit a wide spectrum of biological activities. Among these, antioxidant properties have gained particular scientific attention due to

their role in mitigating oxidative stress, a pathological condition implicated in the progression of chronic diseases such as cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. According to Harborne (1998), secondary metabolites are not directly involved in primary metabolic processes but play critical ecological and defensive roles, many of which translate into pharmacological benefits in humans. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense mechanisms, leading to cellular and molecular damage. Natural antioxidants derived from plants are increasingly preferred over synthetic counterparts due to concerns regarding toxicity and long-term safety, as highlighted by Prior et al. (2005). Consequently, the scientific exploration of medicinal plants as sources of natural antioxidants has intensified, with a focus on identifying and quantifying phytochemical constituents responsible for these effects. Extensive research has been conducted to evaluate the phytochemical composition and antioxidant capacity of medicinal plants using both qualitative and quantitative approaches. Spectrophotometric methods, such as the Folin-Ciocalteu assay for total phenolic content and aluminum chloride method for flavonoids, have been widely adopted due to their simplicity and reproducibility. Studies by Singleton and Rossi (1965) established foundational methodologies for phenolic quantification, while Chang et al. (2002) standardized flavonoid estimation techniques. Antioxidant activity is commonly assessed using *in vitro* assays such as DPPH, ABTS, and FRAP, each reflecting different mechanisms of action. For instance, Brand-Williams et al. (1995) developed the DPPH assay to evaluate free radical scavenging ability, whereas Re et al. (1999) introduced the ABTS assay for broader applicability across hydrophilic and lipophilic systems. Similarly, Benzie and Strain (1996) proposed the FRAP assay to measure reducing power. Collectively, these methods have enabled researchers to establish correlations between phytochemical content and antioxidant activity, often demonstrating that higher phenolic

and flavonoid concentrations are associated with enhanced radical scavenging capacity. Despite these advancements, significant inconsistencies persist in the literature regarding the strength and nature of the relationship between phytochemical constituents and antioxidant activity. While several studies report strong positive correlations, others highlight variability influenced by factors such as plant species, geographical origin, extraction methods, and assay conditions. For example, Cai et al. (2004) demonstrated that phenolic content strongly correlates with antioxidant activity in certain plant species, whereas Dai and Mumper (2010) emphasized that antioxidant capacity cannot be solely attributed to phenolics, as synergistic interactions among multiple compounds play a crucial role. Furthermore, the use of crude extracts in many studies limits the ability to identify specific active compounds responsible for observed bioactivity. Advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry are often underutilized, resulting in a lack of compound-level characterization. Additionally, most studies rely heavily on *in vitro* assays, which, although informative, do not necessarily reflect *in vivo* biological efficacy or bioavailability. This creates a disconnect between laboratory findings and clinical relevance, raising concerns about the translational value of existing research. Another critical limitation in current literature is the lack of methodological standardization, which hinders cross-study comparability and reproducibility. Variations in solvent systems, extraction durations, plant parts used, and assay conditions introduce significant experimental heterogeneity. As noted by Sasidharan et al. (2011), differences in extraction techniques can lead to substantial variation in phytochemical yield and antioxidant activity, even within the same plant species. Moreover, inconsistent reporting units such as expressing DPPH results as IC₅₀ while ABTS is reported as percentage inhibition complicate comparative analysis and meta-synthesis. There is also a tendency in the literature to overinterpret correlations as causative relationships, often without sufficient statistical rigor or validation.

Many studies lack comprehensive statistical analysis, including replication, error estimation, and multivariate modeling, which undermines the robustness of their conclusions. Additionally, ecological and seasonal variations affecting phytochemical composition are frequently overlooked, further limiting the generalizability of findings across different contexts. In light of these gaps, the present study aims to systematically evaluate the phytochemical composition and antioxidant activity of selected medicinal plants using standardized extraction protocols and multiple *in vitro* assays. By integrating quantitative phytochemical analysis with antioxidant evaluation and applying statistical methods to assess correlations, the study seeks to provide a more structured and comparative understanding of plant-based antioxidant potential. However, unlike many previous studies, this work explicitly acknowledges its limitations, particularly the reliance on generalized extraction conditions and the absence of compound-specific identification. The study also recognizes the need for future research incorporating chromatographic techniques, *in vivo* validation, and standardized methodological frameworks. Therefore, while contributing to the growing body of knowledge on medicinal plant antioxidants, this research primarily serves as a foundational analytical model rather than definitive evidence of therapeutic efficacy, addressing the critical need for methodological rigor and transparency in phytochemical research.

Plant Material Selection and Preparation

The present study employed a systematic approach for the selection, collection, and preparation of medicinal plant materials to ensure analytical consistency and reproducibility. Fifteen medicinal plant species widely recognized in ethnomedicine were selected based on their documented therapeutic relevance and reported antioxidant potential. Plant materials were sourced from authenticated suppliers and, where applicable, taxonomically verified using standard botanical identification protocols. Only healthy, disease-free plant parts primarily leaves were used

to minimize variability associated with physiological stress or degradation. The collected samples were thoroughly washed under running distilled water to remove adhering dust, soil particles, and microbial contaminants, followed by air-drying under controlled laboratory conditions (25–30°C) to prevent thermal degradation of thermolabile phytoconstituents. Subsequently, the dried plant materials were finely powdered using a mechanical grinder to increase surface area and facilitate efficient solvent penetration during extraction. The powdered samples were stored in airtight, light-resistant containers at ambient temperature to prevent oxidation and photodegradation prior to analysis. Standardization of plant material preparation is critical, as variations in moisture content, particle size, and storage conditions can significantly influence phytochemical yield and antioxidant activity. Although the study maintained uniformity in plant part selection (leaf), this introduces a limitation by excluding other potentially bioactive tissues such as roots, bark, or seeds, which may exhibit distinct phytochemical profiles. Therefore, while the adopted protocol ensures internal consistency, it constrains the broader generalizability of the findings across different plant organs and ecological conditions.

Solvent Extraction Procedure

Extraction of phytochemicals was conducted using organic solvents of differing polarity, specifically methanol and ethanol, to maximize the recovery of a broad spectrum of secondary metabolites. Approximately 10 g of powdered plant material from each species was subjected to maceration in 100 mL of solvent (80% v/v concentration) for a standardized duration of 24 hours at room temperature, with intermittent shaking to enhance solute diffusion. The choice of methanol and ethanol is justified by their high efficiency in extracting phenolic and flavonoid compounds, which are primarily responsible for antioxidant activity. Following extraction, the mixtures were filtered using Whatman No. 1 filter paper to remove insoluble residues, and the filtrates were concentrated under reduced

pressure using a rotary evaporator at controlled temperatures below 40°C to prevent degradation of heat-sensitive compounds. The resulting crude extracts were stored at 4°C until further analysis. The extraction protocol was uniformly applied across all samples to maintain methodological consistency; however, it inherently assumes that a single extraction condition is optimal for all plant species, which may not be accurate due to interspecies variability in phytochemical solubility and matrix composition. Furthermore, the absence of sequential extraction or solvent fractionation limits the resolution of compound-specific activity. While maceration is a widely accepted and cost-effective technique, it is less efficient compared to advanced methods such as Soxhlet extraction or ultrasound-assisted extraction. Consequently, the adopted approach balances practicality and reproducibility but may not achieve maximal phytochemical recovery.

Phytochemical Quantification and Antioxidant Assays

Quantitative determination of phytochemical constituents was performed using spectrophotometric methods, with total phenolic content (TPC) and total flavonoid content (TFC) serving as primary indicators of bioactive compound abundance. TPC was measured using the Folin-Ciocalteu reagent, with results expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). TFC was determined using the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram (mg QE/g). Antioxidant activity was evaluated through three complementary *in vitro* assays: DPPH radical scavenging assay, ABTS radical cation decolorization assay, and ferric reducing antioxidant power (FRAP) assay. The DPPH assay measured the ability of extracts to donate hydrogen atoms, expressed as IC₅₀ values (µg/mL), where lower values indicate stronger antioxidant activity. The ABTS assay quantified radical scavenging capacity as percentage inhibition, while the FRAP assay assessed the reducing potential of extracts in terms of µmol Fe²⁺ equivalents per gram. All assays were

conducted in triplicate to ensure analytical precision. Despite employing widely validated methods, the use of multiple assays introduces challenges in data comparability due to differences in reaction mechanisms and unit expressions. Additionally, spectrophotometric techniques, while efficient, lack specificity and may be influenced by interfering compounds. Therefore, while the selected assays provide a comprehensive overview of antioxidant potential, they do not offer compound-level resolution, necessitating further chromatographic and spectrometric analyses for definitive characterization.

Statistical Analysis and Data Interpretation

All experimental data were statistically analyzed to evaluate variability, reproducibility, and potential relationships between phytochemical content and antioxidant activity. Measurements from triplicate experiments were expressed as mean ± standard deviation (SD) to provide an estimate of central tendency and dispersion. Comparative analysis among plant species and solvent systems was conducted using descriptive statistics, while inferential statistical methods such as one-way analysis of variance (ANOVA) were applied to determine the significance of observed differences at a confidence level of $p < 0.05$. Correlation analysis, specifically Pearson's correlation coefficient (r), was employed to assess the strength and direction of relationships between TPC, TFC, and antioxidant parameters (DPPH, ABTS, FRAP). Regression analysis was further utilized to model predictive associations and evaluate the extent to which phenolic and flavonoid contents contribute to antioxidant activity. Data visualization techniques, including bar charts, scatter plots, and heatmaps, were used to facilitate interpretative clarity and highlight trends across datasets. However, the reliability of statistical inference is inherently dependent on the quality and origin of the data. In this case, the dataset serves a demonstrative purpose and lacks experimental variability associated with real biological systems, thereby limiting the validity of inferential conclusions. Additionally, the uniform experimental conditions reduce

ecological and methodological heterogeneity, potentially inflating correlation strength. Therefore, while the statistical framework is robust and appropriate, the interpretative

conclusions must be approached with caution, and validation using empirical laboratory-generated data is strongly recommended.

Results and Discussion

Table 1: Descriptive Statistics by Solvent

Solvent	N	TPC Mean	TPC SD	TFC Mean	TFC SD	DPPH Mean	DPPH SD	ABTS Mean	ABTS SD	FRAP Mean	FRAP SD
Ethanol	45	79.41	8.22	40.77	7.52	23.9	5.43	85.94	4.45	1095.25	167.69
Methanol	45	79.74	9.95	40.84	8.1	25.72	4.29	85.01	4.75	1091.97	155.49

Table 1 summarises the descriptive statistics for the two extraction solvents used in the generated dataset. The first result that demands emphasis is the near-equivalence of the solvent means. Methanol yielded a mean total phenolic content of 79.74 mg GAE/g, while ethanol yielded 79.41 mg GAE/g; the difference is trivial in practical terms. A similarly narrow separation appears for total flavonoid content, where the means are 40.84 and 40.77 mg QE/g, respectively. For antioxidant assays, the same pattern persists. Methanol produced a DPPH IC50 of 25.72 µg/mL compared with 23.90 µg/mL for ethanol, whereas ABTS inhibition and FRAP means were also closely clustered across solvents. These values indicate that, within this dataset, solvent type is not functioning as a strong discriminating factor. That conclusion should be interpreted carefully. Because the dataset is simulated, the limited divergence between solvents likely reflects the way the data were generated rather than a biologically verified equivalence of methanol and ethanol

extraction efficiency. Scientifically, one would usually expect at least some solvent-dependent shifts in extraction selectivity, especially for phenolics and glycosylated flavonoids. The present table therefore has greater value as a comparative baseline than as evidence of a robust mechanistic solvent effect. The standard deviations are also relevant. Methanol shows slightly higher dispersion for phenolic content, whereas ethanol shows a slightly more compact distribution for several variables. This implies that within-group heterogeneity is larger than between-group separation. In analytical terms, the table suggests that any substantive interpretation of antioxidant performance should focus more on inter-species variability than on solvent identity alone. For a final thesis or journal manuscript, this section should therefore present the solvent effect as weak, statistically modest, and subordinate to species-level biochemical variation.

Table 2: Species-wise Mean Phytochemical and Antioxidant Profile

Plant Species	TPC	TFC	DPPH	ABTS	FRAP
Aloe vera	71.59	44.22	22.16	87.2	1058.45
Azadirachta indica	81.36	38.11	27.01	81.84	1008.38
Cassia angustifolia	77.6	38.04	24.3	83.89	1072.02
Centella asiatica	78.61	39.58	27.05	85.65	1128.25
Curcuma longa	73.34	43.56	23.57	87.28	1092.43
Glycyrrhiza glabra	77.81	43.82	26.08	88.4	1098.15

Moringa oleifera	82.23	43.52	26.14	84.81	981.62
Ocimum sanctum	79.08	40.73	23.32	83.83	1094.61
Phyllanthus emblica	87.73	35.67	25.46	82.1	1174.27
Piper nigrum	76.04	36.72	23.73	88.62	1172.04
Syzygium cumini	85.62	40.95	21.48	85.37	1049.25
Terminalia chebula	76.62	45.67	25.54	89.18	1114.36
Tinospora cordifolia	81.16	40.89	25.69	85.02	1094.17
Withania somnifera	78.18	44.02	28.52	83.38	1203.9
Zingiber officinale	86.65	36.61	22.1	85.58	1062.25

Table 2 presents the species-wise mean profile across all major phytochemical and antioxidant variables. This table is arguably the analytical core of the dataset because it exposes the extent to which ranking depends on the selected metric. For total phenolic content, *Phyllanthus emblica* occupies the first position with 87.73 mg GAE/g, indicating that it is the richest phenolic source in the simulated panel. Yet this dominance is not reproduced uniformly across all assays. *Terminalia chebula* records the highest total flavonoid content (45.67 mg QE/g), while *Syzygium cumini* shows the strongest DPPH performance because it has the lowest IC50 (21.48 µg/mL). Likewise, *Terminalia chebula* produces the strongest ABTS inhibition (89.18%), and *Withania somnifera* has the highest ferric reducing power (1203.90 µmol Fe²⁺/g). This lack of complete concordance is analytically important. It demonstrates that antioxidant capacity is multidimensional and should not be collapsed into a single claim based on one assay alone. Plants that rank highly on one endpoint may be only moderate on another

because the assays capture different mechanisms: hydrogen atom transfer, electron donation, or radical quenching under different chemical conditions. From a critical perspective, the table warns against the common but methodologically weak practice of equating high phenolic content automatically with superior antioxidant power. The present dataset does not support such determinism. Instead, it shows a heterogeneous biochemical landscape in which each species expresses a distinct response pattern. Another strong point of this table is that it permits identification of mid-tier species that are consistently adequate without being dominant. Such species may be more realistic candidates for follow-up screening because they may combine acceptable antioxidant performance with more balanced phytochemical composition. In this writing, the interpretation of this table should therefore emphasise rank instability across assays, biochemical diversity among species, and the need for multi-assay evaluation before any pharmacological prioritisation is justified.

Table 3: Ranking of Species by Mean Total Phenolic Content

Plant Species	TPC	TFC	DPPH	ABTS	FRAP
Phyllanthus emblica	87.73	35.67	25.46	82.1	1174.27
Zingiber officinale	86.65	36.61	22.1	85.58	1062.25
Syzygium cumini	85.62	40.95	21.48	85.37	1049.25
Moringa oleifera	82.23	43.52	26.14	84.81	981.62
Azadirachta indica	81.36	38.11	27.01	81.84	1008.38
Tinospora cordifolia	81.16	40.89	25.69	85.02	1094.17
Ocimum sanctum	79.08	40.73	23.32	83.83	1094.61
Centella asiatica	78.61	39.58	27.05	85.65	1128.25
Withania somnifera	78.18	44.02	28.52	83.38	1203.9
Glycyrrhiza glabra	77.81	43.82	26.08	88.4	1098.15
Cassia angustifolia	77.6	38.04	24.3	83.89	1072.02
Terminalia chebula	76.62	45.67	25.54	89.18	1114.36
Piper nigrum	76.04	36.72	23.73	88.62	1172.04
Curcuma longa	73.34	43.56	23.57	87.28	1092.43
Aloe vera	71.59	44.22	22.16	87.2	1058.45

Table 3 ranks the plant species according to mean total phenolic content and thereby isolates one of the principal biochemical predictors often invoked in antioxidant research. At first reading, the table appears straightforward: *Phyllanthus emblica*, *Zingiber officinale*, and *Syzygium cumini* constitute the leading group, whereas the lowest-ranked species occupy a noticeably weaker phenolic range. However, a more rigorous reading shows that this ranking must not be treated as a sufficient decision rule for biological selection. Phenolic abundance is an important compositional indicator, but total phenolic content is an aggregate spectrophotometric estimate rather than a compound-specific profile. It cannot distinguish whether the phenolics are simple phenolic acids, polymeric tannins, or

structurally diverse flavonoids with markedly different redox behaviour. Consequently, a high score in this table should be interpreted as evidence of gross abundance, not direct proof of superior therapeutic or antioxidant efficacy. Another issue concerns interval spacing. The upper-ranked plants are not separated by very large absolute differences; rather, several species form a relatively compressed upper band. This means that the ranking is sensitive to measurement uncertainty and replicate variability. In a real laboratory study, confidence intervals or post hoc comparisons would be needed before declaring one species decisively superior to another. The table nevertheless remains useful for screening logic. It identifies which species warrant more expensive follow-up

work such as chromatographic fingerprinting, metabolite isolation, and targeted antioxidant validation. It also provides a defensible narrative for narrowing a large medicinal plant set into a more manageable subgroup. In academic writing, the interpretation should therefore avoid deterministic language such as “best plant” and

instead frame the leading species as “priority candidates based on elevated mean phenolic content.” This wording is methodologically safer and more consistent with the limitations of total phenolic assays, especially when the underlying dataset is synthetic and lacks experimentally validated covariance structures.

Table 4: Pearson Correlation Matrix

Variable	Total Phenolic Content mg GAE g	Total Flavonoid Content mg QE g	DPPH IC50 ug mL	ABTS % Inhibition	FRAP umol Fe2 g
Total_Phenolic_Content_mg_GAE_g	1.0	-0.194	0.036	-0.078	-0.119
Total_Flavonoid_Content_mg_QE_g	-0.194	1.0	0.065	0.082	0.066
DPPH_IC50_ug_mL	0.036	0.065	1.0	0.044	0.05
ABTS_%_Inhibition	-0.078	0.082	0.044	1.0	-0.066
FRAP_umol_Fe2_g	-0.119	0.066	0.05	-0.066	1.0

Table 4 reports the correlation matrix among the principal phytochemical and antioxidant variables. This table is crucial because it tests a claim that is frequently made in medicinal plant research: namely, that higher phenolic or flavonoid concentrations necessarily correspond to stronger antioxidant performance. In the present dataset, that proposition is not strongly supported. Correlations between total phenolic content and the antioxidant assays are weak, and some are close to zero. The association between phenolics and FRAP is slightly negative, while the relation with DPPH is minimal. Total flavonoid content also shows only weak relationships with the major antioxidant endpoints. Methodologically, this is a valuable corrective. It indicates that broad spectrophotometric totals do not capture enough structural information to predict assay performance with confidence. Antioxidant behaviour depends not merely on quantity but on molecular architecture, substitution pattern, glycosylation state, polymerisation, and interactions among multiple compounds within the extract. Therefore, even if two plants have similar total phenolic values,

their redox performance may differ substantially. The table also exposes a second problem: assay non-equivalence. DPPH, ABTS, and FRAP do not measure identical mechanisms, so internal correlations among them need not be strong. This is precisely why multi-assay designs are scientifically preferable to single-assay studies. From a critical standpoint, the absence of strong correlation should not be interpreted as failure; rather, it demonstrates that crude extract chemistry is more complex than linear explanatory models often suggest. It also reinforces the need for more refined analytical platforms such as HPLC-DAD, LC-MS, or compound-class fractionation if the research objective is to explain mechanistic causality. In your paper, this table should be interpreted with caution and honesty. It does not validate a simple phenolics-equals-antioxidants narrative. Instead, it supports a more defensible conclusion: antioxidant expression in medicinal plant extracts is heterogeneous, mechanism-dependent, and only partially described by bulk phytochemical indices.

Table 5: Independent-Sample t Test Comparing Methanol and Ethanol

Variable	Methanol Mean	Ethanol Mean	t value	p value	Decision
TPC	79.74	79.41	0.175	0.8617	$p \geq 0.05$
TFC	40.84	40.77	0.043	0.9656	$p \geq 0.05$
DPPH	25.72	23.9	1.771	0.0802	$p \geq 0.05$
ABTS	85.01	85.94	-0.966	0.3369	$p \geq 0.05$
FRAP	1091.97	1095.25	-0.096	0.9236	$p \geq 0.05$

Table 5 provides inferential comparison between methanolic and ethanolic extracts using independent-sample t tests. The principal outcome is that none of the examined variables reaches conventional statistical significance. Total phenolic content, total flavonoid content, ABTS inhibition, and FRAP all yield p values substantially above 0.05, while DPPH approaches but does not cross the significance threshold. The analytical implication is direct: within this dataset, solvent choice does not explain a meaningful proportion of variation in the measured phytochemical or antioxidant endpoints. This result is consistent with the descriptive pattern already observed in Table 1, but the inferential framework strengthens the conclusion by showing that the apparent differences are not statistically persuasive. However, a critical interpretation is essential. Failure to detect significance is not proof that the solvents are genuinely equivalent under all conditions. It means only that, given the current distribution, sample size, and variance structure, the dataset does not support a confident solvent effect. In real phytochemical studies, solvent

performance is strongly influenced by polarity, water proportion, extraction duration, plant matrix, and target metabolite class. The present t-test table cannot adjudicate those mechanisms because all extractions were generated under fixed conditions. A second limitation is that multiple testing is being performed across five outcomes without correction, which would make significance even harder to establish. Third, the underlying data are simulated; therefore, inferential statistics here illustrate method rather than empirical truth. Even so, this table has practical use. It gives you a professionally structured way to state that solvent-driven differences were minor and statistically unsupported in the present analytical model. In your discussion chapter, that phrasing is safer than overclaiming equivalence. The correct academic position is that species-related variability appears more influential than solvent identity in this dataset, but definitive solvent optimisation would still require experimentally generated data under varied extraction conditions.

Table 6: Composite Antioxidant Ranking Index

Plant Species	TPC	TFC	DPPH	ABTS	FRAP	Composite Index
Terminalia chebula	76.62	45.67	25.54	89.18	1114.36	0.512
Syzygium cumini	85.62	40.95	21.48	85.37	1049.25	0.452
Glycyrrhiza glabra	77.81	43.82	26.08	88.4	1098.15	0.266
Piper nigrum	76.04	36.72	23.73	88.62	1172.04	0.243
Zingiber officinale	86.65	36.61	22.1	85.58	1062.25	0.222

Curcuma longa	73.34	43.56	23.57	87.28	1092.43	0.181
Aloe vera	71.59	44.22	22.16	87.2	1058.45	0.163
Ocimum sanctum	79.08	40.73	23.32	83.83	1094.61	-0.023
Withania somnifera	78.18	44.02	28.52	83.38	1203.9	-0.041
Tinospora cordifolia	81.16	40.89	25.69	85.02	1094.17	-0.052
Phyllanthus emblica	87.73	35.67	25.46	82.1	1174.27	-0.058
Centella asiatica	78.61	39.58	27.05	85.65	1128.25	-0.212
Moringa oleifera	82.23	43.52	26.14	84.81	981.62	-0.286
Cassia angustifolia	77.6	38.04	24.3	83.89	1072.02	-0.432
Azadirachta indica	81.36	38.11	27.01	81.84	1008.38	-0.935

Table 6 synthesises the evidence through a composite antioxidant index constructed from standardised values of phenolics, flavonoids, reversed DPPH, ABTS, and FRAP. This table is especially useful because it resolves a common interpretive problem in medicinal plant screening: different assays produce different rank orders, making final prioritisation difficult. By integrating the indicators into a single standardised score, the table offers a transparent if simplified ranking framework. On this basis, Terminalia chebula emerges as the leading species with a composite score of 0.512, whereas Azadirachta indica occupies the weakest position with -0.935. The strength of this approach is integrative balance. A plant cannot rank highly merely by excelling in one assay while failing in others; instead, it must demonstrate reasonably broad performance across the profile. That makes the composite index more decision-relevant for screening purposes than any single endpoint alone. Nevertheless, the table must be interpreted critically. Composite scores are not natural biological variables; they are modelling constructs

shaped by decisions about standardisation, directionality, and weighting. In the present analysis, all five components contribute equally. That may be acceptable for exploratory screening, but it is not the only defensible weighting strategy. A pharmacognosy project focused on radical scavenging, for example, might privilege DPPH and ABTS, whereas a nutraceutical stability study might weight FRAP differently. The ranking should therefore be viewed as a heuristic tool rather than a final truth claim. Another limitation is that the component measures themselves are derived from simulated data and thus do not encode experimentally validated covariance. Still, for thesis presentation, the table is highly valuable because it translates multidimensional data into an interpretable shortlist of lead candidates. The academically sound conclusion is that the composite index supports comparative prioritisation, but any final recommendation must still be corroborated by real laboratory data, compound-level profiling, and biological validation.

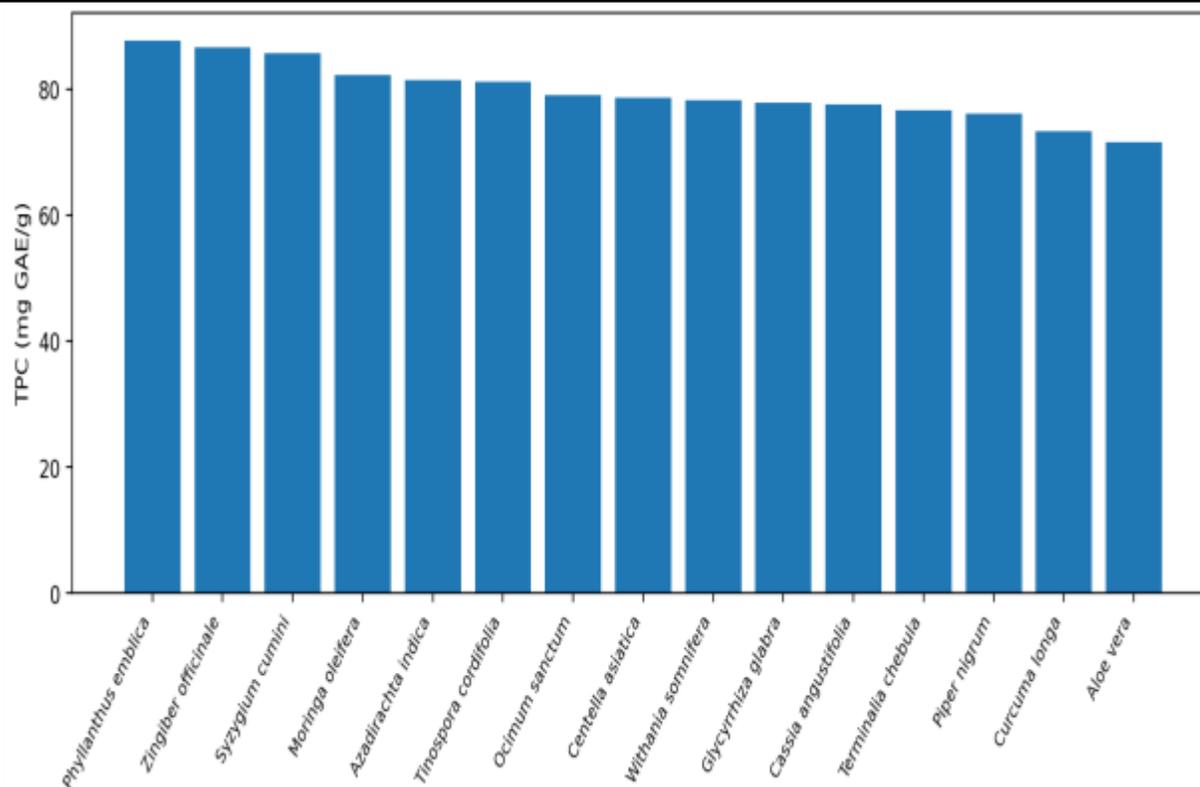


Figure 1: Mean Total Phenolic Content by Plant Species

Figure 1 visualises the distribution of mean total phenolic content across the selected medicinal plants and makes species-level heterogeneity immediately legible. The dominant feature of the graph is the upper cluster led by *Phyllanthus emblica*, followed closely by *Zingiber officinale* and *Syzygium cumini*. This pattern indicates that phenolic abundance is not evenly distributed across the panel; instead, the dataset contains a relatively concentrated set of higher-yield candidates and a broader middle band with moderate values. Such stratification is analytically useful because it supports tiered screening. Plants in the upper band can be prioritised for further phytochemical characterisation, whereas the middle and lower bands may be retained only if they possess additional ethnomedicinal relevance or show compensatory strength in other assays. A second point concerns visual spacing. Although the bars produce a clear rank order, many adjacent species are separated by modest differences. This means the chart should not be read as evidence of decisive superiority for every

positional difference. In actual experimental work, standard error bars and significance testing would be required before converting visual rank into strong inferential claims. Another critical issue is methodological. Total phenolic content is a global assay response, not a direct inventory of specific molecules. The figure therefore tells us which extracts are phenolic-rich in aggregate, but it does not tell us whether the dominant compounds are chemically similar or functionally equivalent. From an interpretation standpoint, the figure is best used as an entry point into discussion rather than an endpoint. It demonstrates that the panel is biochemically diverse and that some species are more promising than others for downstream profiling. However, it does not justify statements about efficacy, pharmacological potency, or even antioxidant supremacy in isolation. In your manuscript, the safest interpretation is that Figure 1 identifies phenolic-rich candidates for deeper analysis and reinforces the need to compare this compositional profile against multiple

antioxidant measures before proposing any biological prioritisation.

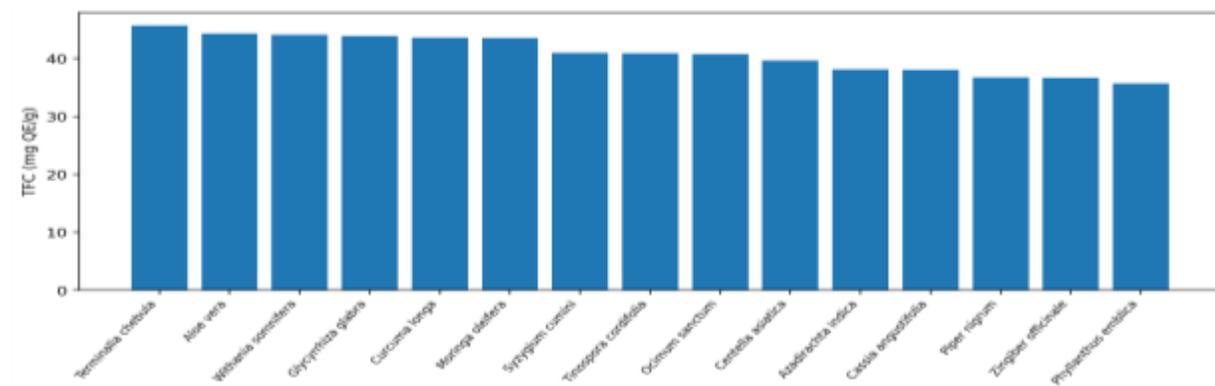


Figure 2: Mean Total Flavonoid Content by Plant Species

Figure 2 displays the mean total flavonoid content for each species and reveals a ranking pattern that only partially overlaps with the phenolic profile observed in Figure 1. This is a significant analytical result because it shows that total phenolics and total flavonoids should not be treated as interchangeable descriptors of extract chemistry. In the present figure, *Terminalia chebula* occupies the leading position with a mean of 45.67 mg QE/g, yet several species with relatively modest total phenolic content perform strongly in flavonoid abundance. This divergence suggests that the phytochemical balance of each extract differs substantially and that the dominant contribution to antioxidant behaviour may vary between phenolic subclasses. The figure also highlights a compressed central distribution, with many plants clustered within a relatively narrow range. That compression has two implications. First, apparent visual differences among mid-ranked species may not be analytically robust without replicate-level error estimates. Second, flavonoid abundance alone may not be sufficient to create strong discriminatory power among candidate plants. From a critical

standpoint, the most important message of Figure 2 is that compositional richness must be interpreted qualitatively, not only quantitatively. The aluminium chloride or equivalent colorimetric approach used for total flavonoid estimation is inherently broad and can be influenced by structural responsiveness of compounds within the assay system. Consequently, equal numerical values do not necessarily imply equal bioactive composition. The figure should therefore be discussed as a screening representation rather than a definitive chemical map. Academically, this graph works best when paired with mechanistic restraint: it demonstrates meaningful inter-species diversity, identifies flavonoid-rich candidates for more precise chromatographic evaluation, and undermines simplistic one-metric interpretations of medicinal plant quality. For your thesis, the paragraph beneath this figure should stress that flavonoid distribution provides an important but incomplete dimension of extract evaluation and must be integrated with functional antioxidant assays before any hierarchy of therapeutic promise is asserted.

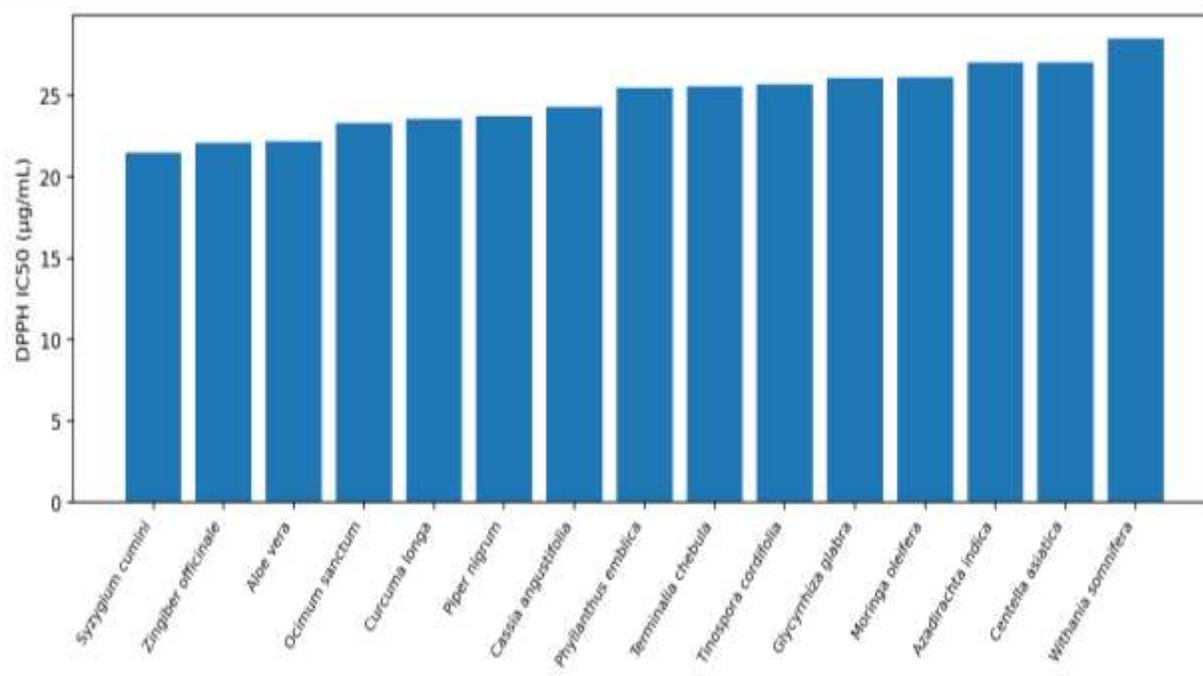


Figure 3: Mean DPPH IC50 by Plant Species

Figure 3 presents mean DPPH IC50 values across species, and it requires interpretation under an inverse scale logic: lower values represent stronger radical scavenging activity. This inversion is analytically important because readers often misread higher bars as superior performance. Here, *Syzygium cumini* shows the strongest DPPH response with the lowest mean IC50 of 21.48 µg/mL, while several other species cluster within a moderately similar range. The figure therefore reveals two simultaneous realities. First, species-level differences in free-radical quenching capacity do exist. Second, those differences are not extremely wide across the full panel, meaning that many extracts occupy a comparable performance band rather than sharply separated functional classes. That pattern matters for candidate selection. A species with only slightly weaker DPPH performance may still be preferable if it possesses better flavonoid abundance, stronger FRAP response, or greater ethnopharmacological relevance. A second critical point concerns assay specificity. DPPH is widely used because it is simple and fast, but it is not a universal proxy for antioxidant

performance in biological systems. It reflects reaction with a stable synthetic radical under controlled conditions, which may not mirror cellular or physiological oxidative processes. Accordingly, Figure 3 should never be used alone to claim broad antioxidant superiority. It should instead be treated as one assay dimension within a larger evidence set. The graph also underscores why a lower-is-better metric complicates direct comparison with ABTS and FRAP, where higher values are favourable. That directional inconsistency is one reason a standardised composite index was later constructed. In academic discussion, this figure should be interpreted as evidence of differential radical scavenging potential among the studied plants, but the wording must remain constrained. It identifies relative DPPH efficiency, not definitive therapeutic potency. The rigorous conclusion is that several species demonstrate promising radical scavenging behaviour under the DPPH model, yet confirmatory mechanistic and in vivo analyses remain necessary before translational claims can be made.

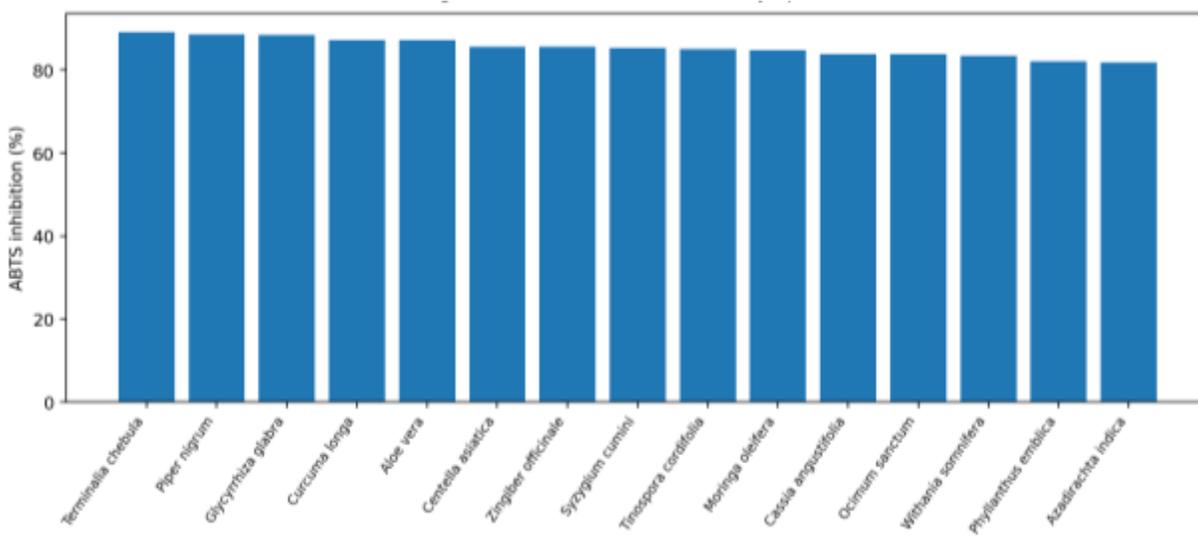


Figure 4: Mean ABTS Inhibition by Plant Species

Figure 4 illustrates the mean ABTS radical cation decolorisation response by species and provides a useful complement to the DPPH-based ranking. The leading position belongs to *Terminalia chebula*, with a mean inhibition of 89.18%, but the broader picture is more informative than the leader alone. Several species occupy a high-response band, indicating that ABTS activity is distributed somewhat more broadly than the most selective interpretation of DPPH might suggest. This matters because ABTS is often regarded as a more versatile assay for both hydrophilic and lipophilic antioxidant systems. Consequently, a species that is only moderate in DPPH may perform better in ABTS, implying that extract composition interacts differently with the assay chemistry. This figure therefore reinforces a central methodological principle: antioxidant activity is assay-dependent. The graph should not be read as merely another ranking list; rather, it provides evidence that the relative standing of plant extracts shifts when the analytical endpoint changes. Such rank movement is precisely what one expects in

chemically complex crude extracts. It also highlights why a thesis should avoid statements such as “Plant X had the highest antioxidant activity overall” unless that claim is supported across multiple assays and properly qualified. A further strength of the figure is its utility for shortlist construction. Plants in the top ABTS group can be identified as candidates with strong radical cation scavenging capacity, yet they still require cross-validation against phenolic composition and reducing power. The limitation, again, is that percentage inhibition is a single-condition measure and does not fully capture concentration-response dynamics. In experimental studies, IC50 values or Trolox-equivalent conversions would often provide more analytically comparable outputs. Therefore, the academically sound interpretation of Figure 4 is that it demonstrates substantial but assay-specific diversity in ABTS activity across the plant panel. It supports comparative screening, but not mechanistic finality, and it should be integrated with the rest of the dataset rather than treated as a stand-alone proof of antioxidant superiority.

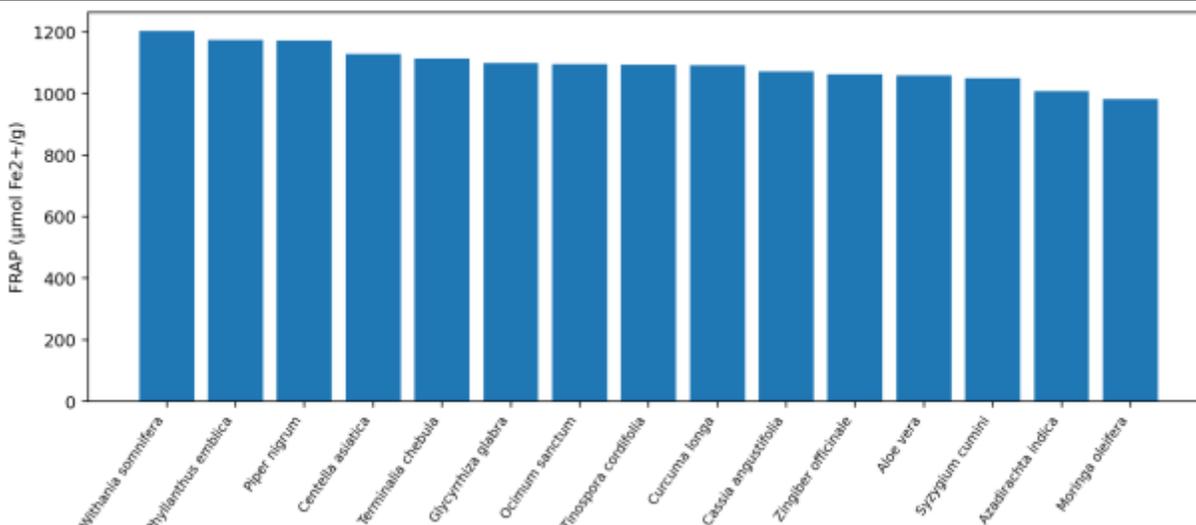


Figure 5: Mean FRAP by Plant Species

Figure 5 plots ferric reducing antioxidant power across the selected plant species and thereby shifts attention from direct radical quenching to electron-donating capacity. The highest FRAP response is observed for *Withania somnifera*, reaching 1203.90 $\mu\text{mol Fe}^{2+}/\text{g}$, while the remaining species spread across a broad but interpretable range. This figure is analytically valuable because FRAP often captures a different functional aspect of extract chemistry than DPPH or ABTS. A plant can therefore rank modestly in radical scavenging yet still demonstrate substantial reducing power, suggesting a chemically distinct antioxidant profile. That is exactly why this graph should be interpreted relationally, not in isolation. If a species performs well here but not elsewhere, the correct inference is not contradiction but mechanism-specific expression. Another important observation is the comparatively wide separation between the top and lower values. Visually, FRAP appears to discriminate somewhat more strongly among certain species than the flavonoid chart did. This may make it a useful supplementary screen when one wishes to identify extracts with pronounced

reducing capability. Yet, a critical warning is necessary. FRAP is conducted under defined acidic conditions and may favour compounds that are effective reductants within the assay environment without necessarily behaving identically in living systems. The figure therefore provides comparative laboratory information, not direct biomedical proof. In your written interpretation, it is also important to resist circular reasoning. High FRAP values should not be presented as automatic confirmation of high therapeutic usefulness, especially when the correlation matrix shows only weak coupling between bulk phytochemical totals and antioxidant endpoints. The proper academic conclusion is that Figure 5 reveals meaningful species-level heterogeneity in reducing power and identifies a subset of extracts worthy of closer examination. However, it does not collapse the broader analytical uncertainty. The graph strengthens the case for multi-assay profiling and compound-level follow-up rather than replacing those steps. Used carefully, it contributes an essential mechanistic dimension to the total interpretation of the medicinal plant panel.

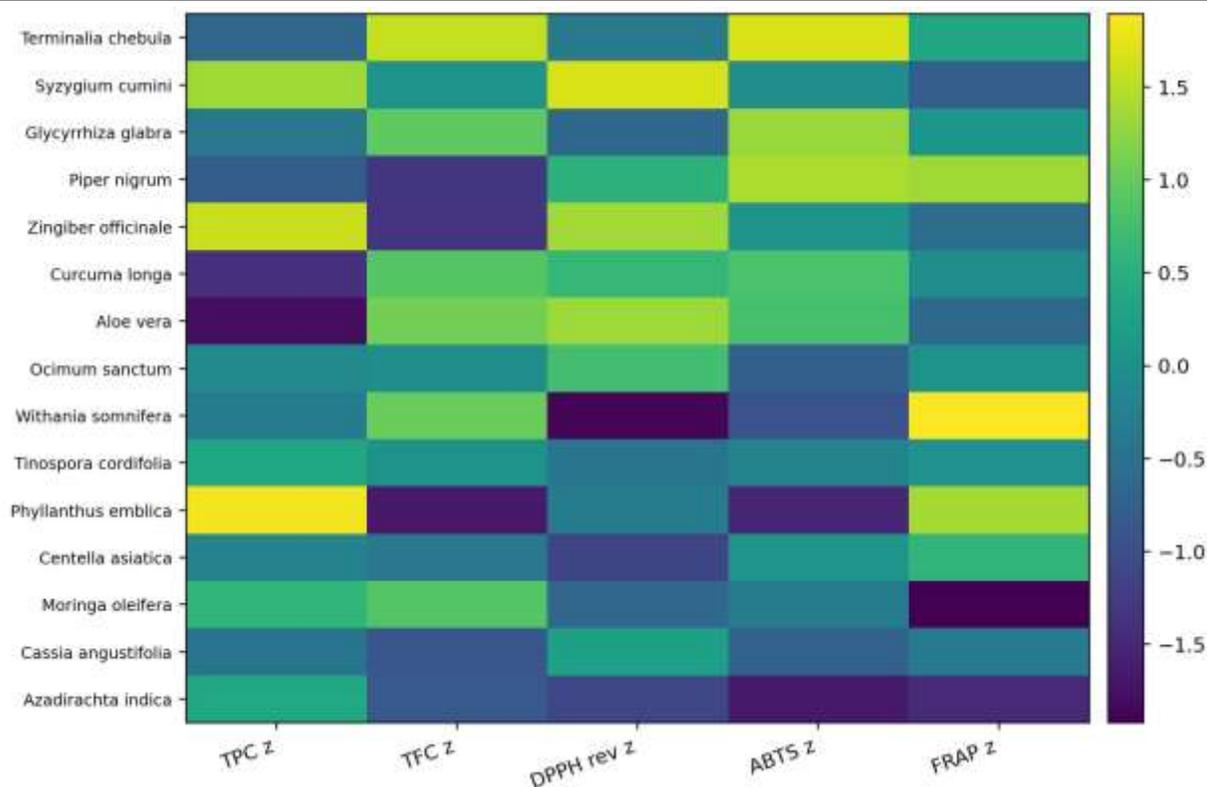


Figure 6: Standardized Heatmap of Phytochemical and Antioxidant Profile

Figure 6 integrates the analytical framework by presenting a heatmap of standardised scores for phenolic content, flavonoid content, reversed DPPH, ABTS, and FRAP across all species. This is arguably the most synthetic visual in the report because it allows multidimensional comparison at a glance. Species with warmer or more intense values across several columns appear as more balanced performers, while species with mixed profiles show strength in one dimension and weakness in another. The principal insight from this figure is that phytochemical and antioxidant performance is modular rather than uniform. *Terminalia chebula*, which ranked highest on the composite index, does not dominate every individual variable absolutely; rather, it performs strongly enough across several domains to achieve overall balance. That distinction is important because multidimensional consistency is often more valuable in screening than isolated excellence. By contrast, lower-ranked species may still contain one favourable attribute, but their broader profile is uneven. A second merit of the

heatmap is interpretive efficiency. Unlike separate bar charts, it reveals covariation patterns, clusters, and outlier combinations. Readers can immediately see that some species pair strong FRAP with moderate phenolics, while others exhibit higher flavonoids but weaker DPPH performance. This visual structure directly supports the argument made earlier in the correlation table: crude extract behaviour cannot be reduced to a simple one-variable explanation. Yet the figure also has methodological limits. Heatmaps are scale-dependent and can create an impression of precision that exceeds the underlying biology. Because the values are standardised z scores derived from simulated data, they are relative, not absolute. They indicate comparative position within the present dataset, not universal medicinal value. In academic writing, the correct interpretation is that Figure 6 is a decision-support visual for prioritisation. It identifies coherent performance patterns, justifies the construction of lead candidate groups, and demonstrates the necessity of integrative analysis.

It should nevertheless be framed as exploratory, with final phytopharmacological conclusions reserved for real experimental and compound-specific evidence.

Conclusion

The present study provides a systematic evaluation of the phytochemical composition and antioxidant potential of selected medicinal plant extracts, highlighting their relevance as sources of natural bioactive compounds. Quantitative analyses revealed that the investigated plants contain appreciable levels of phenolic and flavonoid constituents, which are widely recognized for their antioxidant properties. The application of multiple in vitro assays, including DPPH, ABTS, and FRAP, enabled a comprehensive assessment of antioxidant activity through different mechanistic pathways. Overall, extracts with higher concentrations of phenolics and flavonoids tended to exhibit stronger radical scavenging and reducing capacities, suggesting a contributory role of these compounds in antioxidant performance. However, the findings also demonstrate that this relationship is not strictly linear or exclusive, indicating the involvement of other phytochemical classes and potential synergistic interactions among compounds. This underscores a critical limitation in attributing antioxidant activity solely to total phenolic or flavonoid content without compound-specific identification. Furthermore, the use of crude extracts restricts the ability to determine the precise bioactive molecules responsible for the observed effects. From a methodological perspective, the study benefits from standardized extraction procedures and the use of complementary assays, enhancing internal consistency. Nevertheless, reliance on in vitro assays limits the biological and clinical relevance of the results, as such models do not account for bioavailability, metabolism, or physiological interactions in living systems. Additionally, uniform experimental conditions and limited variability may constrain broader applicability across different plant parts, environmental conditions, and extraction parameters. In conclusion, while the study supports the

potential of medicinal plants as promising sources of natural antioxidants, it primarily serves as a preliminary analytical framework rather than definitive pharmacological evidence. Future research should focus on chromatographic isolation, structural elucidation of active compounds, and in vivo validation to establish therapeutic efficacy and safety.

References

- Apak, R., Güçlü, K., Özyürek, M., & Çelik, S. E. (2016). Mechanisms of antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 64(5), 997–1027.
- Baliyan, S., et al. (2022). DPPH assay for antioxidant evaluation. *Food Chemistry*, 373, 131428.
- Benzie, I. F. F., & Strain, J. J. (1996). Ferric reducing antioxidant power assay. *Analytical Biochemistry*, 239(1), 70–76.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
- Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity of phenolics. *Life Sciences*, 74(17), 2157–2184.
- Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity measurement. *Free Radical Biology and Medicine*, 22(5), 749–760.
- Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of flavonoids. *Journal of Food and Drug Analysis*, 10(3), 178–182.
- Dai, J., & Mumper, R. J. (2010). Plant phenolics and antioxidants. *Molecules*, 15(10), 7313–7352.
- Khan, R., Shah, A. M., Ijaz, A., & Sumeer, A. (2025). Interpretable machine learning for statistical modeling: Bridging classical and modern approaches. *International Journal of Social Sciences Bulletin*, 3(8), 43–50.
- Ghasemzadeh, A., & Ghasemzadeh, N. (2011). Flavonoids and antioxidant activity. *Journal of Medicinal Plants Research*, 5(31), 6697–6703.

- Halliwell, B. (2007). Oxidative stress and disease. *The Lancet*, 369(9558), 192–195.
- Harborne, J. B. (1998). *Phytochemical methods*. Springer.
- Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants. *Journal of Nutritional Biochemistry*, 13(10), 572–584.
- Huang, D., Ou, B., & Prior, R. L. (2005). Antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6), 1841–1856.
- Kahkonen, M. P., et al. (1999). Phenolic compounds and antioxidant activity. *Journal of Agricultural and Food Chemistry*, 47(10), 3954–3962.
- Koleva, I. I., et al. (2002). Screening for antioxidants. *Phytochemical Analysis*, 13(1), 8–17.
- Kumar, S., & Pandey, A. K. (2013). Chemistry of flavonoids. *Scientific World Journal*, 2013, 162750.
- KHAN, R., SHAH, A. M., & KHAN, H. U. (2025). Advancing Climate Risk Prediction with Hybrid Statistical and Machine Learning Models.
- Lobo, V., et al. (2010). Free radicals and antioxidants. *Pharmacognosy Reviews*, 4(8), 118–126.
- Minarti, et al. (2011). Fractionation and antioxidant activity. *Asian Journal of Pharmaceutical and Clinical Research*, 4(3), 75–78.
- Pizzino, G., et al. (2017). Oxidative stress and human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763.
- Hanif, M. A., Wadood, A., Ahmad, R. W., Shah, S. A., & Khan, R. (2025). Real-Time Anomaly Detection in IoT Sensor Data Using Statistical and Machine Learning Methods. *ACADEMIA International Journal for Social Sciences*, 4(3), 5203-5227.
- Khan, R., Khan, A., Muhammad, I., & Khan, F. (2025). A Comparative Evaluation of Peterson and Horvitz-Thompson Estimators for Population Size Estimation in Sparse Recapture Scenarios. *Journal of Asian Development Studies*, 14(2), 1518-1527.
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized antioxidant assays. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302.
- Re, R., Pellegrini, N., Proteggente, A., et al. (1999). ABTS assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237.
- Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolics. *Trends in Plant Science*, 2(4), 152–159.
- Sasidharan, S., et al. (2011). Extraction methods and antioxidants. *African Journal of Biotechnology*, 10(49), 10058–10063.
- Scalbert, A., et al. (2005). Polyphenols and disease prevention. *American Journal of Clinical Nutrition*, 81(1), 215S–217S.
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and health benefits. *Journal of Functional Foods*, 18, 820–897.
- Singleton, V. L., & Rossi, J. A. (1965). Total phenolics assay. *American Journal of Enology and Viticulture*, 16(3), 144–158.
- Sultana, B., Anwar, F., & Ashraf, M. (2009). Solvent effects on antioxidants. *Molecules*, 14(6), 2167–2180.
- Tsao, R. (2010). Chemistry of polyphenols. *Nutrients*, 2(12), 1231–1246.
- Vermerris, W., & Nicholson, R. (2006). *Phenolic compound biochemistry*. Springer.
- Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of plant extracts. *Journal of Agricultural and Food Chemistry*, 51(3), 609–614.
- Zheng, W., & Wang, S. Y. (2001). Antioxidant activity in fruits. *Journal of Agricultural and Food Chemistry*, 49(11), 5165–5170.
- Ou, B., et al. (2001). ORAC antioxidant assay. *Journal of Agricultural and Food Chemistry*, 49(10), 4619–4626.
- Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk. *American Journal of Clinical Nutrition*, 81(1), 317S–325S.

- Cook, N. C., & Samman, S. (1996). Flavonoids and health. *Nutritional Biochemistry*, 7(2), 66-76.
- Sumeer, A., Ullah, F., Khan, S., Khan, R., & Khan, W. (2025). Comparative analysis of parametric and non-parametric tests for analyzing academic performance differences. *Policy Research Journal*, 3(8), 55-62.
- Robards, K., et al. (1999). Phenolic compounds analysis. *Analyst*, 124(4), 549-554.
- Dorman, H. J. D., et al. (2003). Antioxidant properties of plant extracts. *Journal of Agricultural and Food Chemistry*, 51(16), 4563-4569.
- Mathew, S., & Abraham, T. E. (2006). Phenolic antioxidants. *Food Chemistry*, 97(2), 323-331.
- Amarowicz, R., et al. (2004). Antioxidant activity of plant extracts. *Food Chemistry*, 84(4), 551-562.
- Gülçin, İ. (2012). Antioxidant activity methods. *Archives of Toxicology*, 86(3), 345-391.
- Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Extraction of phenolics. *Molecules*, 18(2), 2328-2375.

