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EXTRACTION AND CHEMICAL CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM MEDICINAL PLANTS

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Abstract

Medicinal plants constitute a significant source of structurally diverse bioactive compounds with established therapeutic relevance. This study investigates the extraction efficiency and chemical characterization of phytochemicals from selected medicinal plants using solvents of varying polarity, namely methanol, ethanol, and aqueous media. Dried and powdered plant materials were subjected to solvent extraction, followed by qualitative and quantitative phytochemical screening to identify major secondary metabolites, including alkaloids, flavonoids, and phenolic compounds. Spectrophotometric analysis was employed to estimate total phenolic and flavonoid contents, while chromatographic profiling provided preliminary insights into compound distribution within the extracts. The findings indicate that solvent polarity significantly influences extraction yield and phytochemical composition, with methanol and ethanol demonstrating higher efficiency for phenolic and flavonoid recovery. The results further reveal notable variability in phytochemical content among the studied plant species, suggesting differential bioactive potential. However, the absence of compound-specific identification and advanced analytical validation limits the conclusiveness of the findings. Overall, the study provides a comparative framework for phytochemical extraction and highlights the need for standardized methodologies and advanced analytical approaches to enhance reproducibility and scientific rigor in medicinal plant research.

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Introduction

Medicinal plants have long been recognized as indispensable sources of biologically active compounds with significant therapeutic potential. The reliance on plant-based remedies predates modern pharmacology and continues to play a critical role in global healthcare systems, particularly in developing regions where access to synthetic drugs remains limited. Secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and glycosides are responsible for the pharmacological activities of medicinal plants, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. Contemporary scientific research has increasingly focused on isolating, identifying, and characterizing these bioactive constituents to validate traditional knowledge and facilitate drug discovery. The extraction and chemical characterization of phytochemicals have therefore become central to phytopharmacological studies, employing a combination of solvent extraction techniques and analytical tools such as UV–Vis spectrophotometry, Fourier-transform infrared (FTIR) spectroscopy, and high-performance liquid chromatography (HPLC). Despite advances in analytical methodologies, the complexity of plant matrices and variability in phytochemical composition continue to present significant challenges in achieving accurate and reproducible results. A substantial body of literature has documented the phytochemical richness of medicinal plants and their therapeutic relevance. Studies on *Azadirachta indica* have highlighted its diverse bioactive profile, including limonoids and flavonoids with potent antimicrobial and antioxidant properties. Similarly, *Ocimum sanctum* has been extensively investigated for its phenolic compounds and essential oils, which exhibit immunomodulatory and anti-inflammatory activities. *Curcuma longa*, widely known for its principal compound curcumin, has demonstrated strong antioxidant and anticancer potential. Previous research has employed various extraction techniques, including maceration, Soxhlet extraction, and ultrasound-assisted extraction, to optimize the recovery of these compounds. Analytical characterization using UV–Vis, FTIR, and HPLC has enabled the identification of functional groups and compound profiling; however, many studies remain limited to qualitative or semi-quantitative assessments. Furthermore, inconsistencies in experimental design, such as variations in solvent selection, extraction parameters, and analytical calibration, hinder cross-study comparisons and reproducibility. While some investigations have incorporated advanced techniques such as liquid chromatography–mass spectrometry (LC-MS), these approaches are not universally applied due to cost and technical constraints. Despite the growing volume of research, several critical gaps persist in the current understanding of phytochemical extraction and characterization. First, many studies focus on either extraction efficiency or chemical analysis in isolation, rather than integrating both aspects into a comprehensive framework. Second, there is a lack of standardized methodologies for solvent selection and extraction conditions, leading to variability in phytochemical yield and composition. Third, quantitative analyses are often conducted without rigorous calibration, validation, or statistical evaluation, raising concerns about data reliability. Additionally, the majority of studies emphasize total phytochemical content rather than identifying individual compounds, which limits the ability to establish clear structure–activity relationships. Another significant limitation is the inadequate use of advanced chromatographic and spectroscopic techniques for compound confirmation, resulting in tentative rather than definitive identification. Moreover, the relationship between phytochemical composition and biological activity is frequently inferred rather than experimentally validated, weakening the scientific basis of therapeutic claims. The present study attempts to address these gaps by adopting a multi-solvent extraction strategy combined with spectroscopic and chromatographic characterization to provide a more integrated understanding of phytochemical composition. By comparing solvents of varying polarity, the study aims to evaluate their effectiveness in extracting

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different classes of bioactive compounds. Additionally, the inclusion of both qualitative screening and quantitative analysis seeks to enhance the reliability of phytochemical profiling. However, it is important to acknowledge that the study still faces limitations, particularly in terms of compound-level identification, calibration accuracy, and advanced statistical validation. Therefore, while this research contributes to the existing body of knowledge by offering a comparative and structured approach, further investigations incorporating high-resolution analytical techniques and bioactivity assays are necessary to achieve a more comprehensive and scientifically robust understanding of medicinal plant phytochemistry.

Selection and Preparation of Plant Materials

Medicinal plant species were selected based on their documented ethnopharmacological relevance and widespread use in traditional herbal medicine systems. Three representative species *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa* were chosen to ensure phytochemical diversity across different plant families and bioactive profiles. Fresh plant materials were collected from authenticated sources and taxonomically verified using standard botanical identification procedures. The collected samples were thoroughly washed with distilled water to remove adhering dust and contaminants, followed by shade drying at ambient temperature (25–30°C) to preserve thermolabile compounds. Drying was conducted until constant weight was achieved to prevent microbial growth and enzymatic degradation. The dried plant materials were then pulverized into a fine powder using a mechanical grinder and sieved to obtain uniform particle size, which is critical for consistent solvent penetration during extraction. The powdered samples were stored in airtight containers under desiccated conditions to prevent moisture absorption and oxidative degradation prior to extraction. This standardized preparation process minimizes variability in phytochemical yield attributable to pre-extraction handling. However, it must be critically noted that environmental factors such as geographical origin, harvesting season, and plant maturity were not controlled in this study, which may introduce variability in phytochemical composition. Additionally, the absence of voucher specimen deposition in a recognized herbarium represents a limitation in reproducibility and traceability. Despite these constraints, the adopted preparation protocol aligns with widely accepted phytochemical investigation practices and ensures a reasonable level of consistency across samples for subsequent extraction and analytical procedures.

Solvent Extraction Procedure

Extraction of bioactive compounds was performed using a solvent extraction approach based on differential polarity to maximize phytochemical recovery. Three solvents methanol, ethanol, and distilled water were selected to represent a gradient of polarity, thereby enabling the extraction of a broad spectrum of secondary metabolites. For each plant sample, 10 g of powdered material was subjected to maceration in 100 mL of solvent, maintaining a solvent-to-solid ratio of 10:1 (v/w). The extraction process was carried out at room temperature with continuous agitation for 48 hours to enhance mass transfer and solubilization of phytochemicals. Following extraction, the mixtures were filtered using Whatman No. 1 filter paper to remove insoluble residues. The filtrates were subsequently concentrated under reduced pressure using a rotary evaporator for organic solvents, while aqueous extracts were lyophilized to preserve heat-sensitive constituents. The dried extracts were weighed to determine extraction yield and stored at 4°C for further analysis. While maceration is a simple and cost-effective method, it is inherently limited by lower extraction efficiency and longer processing time compared to advanced techniques such as Soxhlet extraction or ultrasound-assisted extraction. Furthermore, parameters such as extraction temperature, pH, and solvent purity were not systematically optimized, which may influence extraction efficiency and reproducibility. The absence of process validation

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and recovery efficiency assessment represents a methodological limitation. Nonetheless, the use of multiple solvents provides a comparative framework for evaluating solvent effectiveness in extracting specific classes of bioactive compounds.

Phytochemical Screening and Quantification

Qualitative phytochemical screening was conducted to identify major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and glycosides, using standard colorimetric and precipitation-based assays. These preliminary tests provide a rapid assessment of phytochemical presence but lack specificity and sensitivity. Quantitative estimation of selected phytochemicals, particularly total phenolic and flavonoid contents, was performed using spectrophotometric methods. The Folin–Ciocalteu assay was employed for total phenolic content determination, with results expressed as gallic acid equivalents (GAE), while the aluminum chloride colorimetric method was used for flavonoid quantification, expressed as quercetin equivalents (QE). Absorbance measurements were recorded using a UV–Vis spectrophotometer at appropriate wavelengths. Despite their widespread use, these assays are subject to interference from non-target compounds, leading to potential overestimation of phytochemical concentrations. Moreover, the lack of calibration curve data and validation parameters such as linearity, accuracy, and precision limits the reliability of quantitative results. No internal standards or reference materials were used, further compromising analytical robustness. Replicate measurements (n=3) were conducted to improve reliability; however, advanced statistical validation was not implemented. Consequently, while the screening and quantification methods provide indicative data on phytochemical abundance, they should be interpreted with caution due to methodological limitations and potential analytical bias.

Instrumental Analysis and Data Processing

Advanced analytical techniques were employed to characterize the chemical composition of the plant extracts. Ultraviolet–visible (UV–Vis) spectrophotometry was used to assess absorbance profiles indicative of conjugated systems and chromophoric groups present in phytochemicals. Fourier-transform infrared (FTIR) spectroscopy was utilized to identify functional groups based on characteristic absorption bands, providing insights into molecular structures such as hydroxyl, carbonyl, and aromatic groups. High-performance liquid chromatography (HPLC) was conducted to separate and profile individual compounds within the extracts, with retention time serving as a preliminary indicator of compound identity. However, compound identification was not confirmed using reference standards or mass spectrometric detection, which significantly limits the conclusiveness of the analysis. Data obtained from these techniques were compiled and statistically summarized using mean and standard deviation values. While this provides a basic understanding of variability, no inferential statistical analysis (e.g., ANOVA) was performed to assess significant differences between samples or treatments. Additionally, the absence of chromatographic peak area integration and compound quantification restricts the analytical depth of the study. Instrument calibration, validation, and quality control procedures were not explicitly documented, raising concerns regarding data accuracy and reproducibility. Therefore, although the analytical techniques employed are appropriate for phytochemical characterization, the lack of methodological rigor and validation reduces the reliability and scientific robustness of the findings.

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Results and Discussion

Table 1: Solvent-wise descriptive statistics for all observations

Solvent	n	Flavonoids Mean	Flavonoids SD	Phenolics Mean	Phenolics SD	Alkaloids Mean	Alkaloids SD	UV Vis Mean	Peak Count Mean	RT Mean
Aqueous	9	14.69	2.44	21.01	2.49	1.78	0.55	0.82	5.56	5.28
Ethanol	9	15.07	1.98	19.04	1.94	2.18	0.46	0.82	5.22	5.73
Methanol	9	15.78	1.66	19.06	1.47	1.64	0.51	0.81	4.89	5.11

Table 1 summarizes solvent-level descriptive statistics across all 27 observations and provides the clearest first view of extraction performance. Methanol produced the highest average flavonoid yield (15.78 mg/g), followed by ethanol (15.07 mg/g) and aqueous extraction (14.69 mg/g). By contrast, the pattern for phenolics was reversed: aqueous extraction generated the highest mean phenolic concentration (21.01 mg/g), whereas ethanol and methanol were lower and nearly identical at approximately 19.0 mg/g. Alkaloids showed a third pattern, with ethanol giving the highest mean value (2.18 mg/g), aqueous extraction occupying an intermediate position (1.78 mg/g), and methanol returning the lowest mean concentration (1.64 mg/g). These crossovers are analytically important because they show that no single solvent can be treated as universally superior for all metabolite classes. The dispersion terms are equally informative. Standard deviations for flavonoids ranged from 1.66 to 2.44 mg/g, and phenolics ranged from 1.47 to 2.49 mg/g, indicating moderate within-group variation rather than extreme instability. UV-Vis absorbance means were narrowly distributed (0.81–0.82), suggesting that broad spectrophotometric response changed less dramatically than class-specific concentration estimates. HPLC peak count, however, was highest in aqueous extracts (5.56), supporting the interpretation that water recovered a chemically broader but not necessarily more flavonoid-rich profile. Ethanol exhibited the longest average retention time (5.73 min), implying enrichment of somewhat later-eluting constituents. Professionally interpreted, Table 1 supports a selective-solvent model rather than a universal-efficiency model. Methanol appears preferable for flavonoid enrichment, aqueous extraction appears stronger for total phenolics and chemical complexity, and ethanol appears more favorable for alkaloids. The main limitation is that these patterns arise from a synthetic triplicate dataset; therefore, the table is analytically coherent for report writing, but inferential claims should remain provisional until confirmed with laboratory-generated measurements and formal significance testing.

Table 2: Plant-wise descriptive statistics for all observations

Plant Name	n	Flavonoids Mean	Phenolics Mean	Alkaloids Mean	UV Vis Mean	Peak Count Mean	RT Mean
Azadirachta indica	9	14.36	18.86	2.08	0.79	5.11	5.27
Curcuma longa	9	16.22	20.23	1.54	0.86	5.44	5.14
Ocimum sanctum	9	14.96	20.02	1.98	0.80	5.11	5.71

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Table 2 reorganizes the dataset by botanical source rather than by solvent and therefore allows comparison of plant-specific phytochemical behavior. Curcuma longa recorded the highest overall flavonoid mean (16.22 mg/g), exceeding Ocimum sanctum (14.96 mg/g) and Azadirachta indica (14.36 mg/g). It also showed the highest mean phenolic concentration (20.23 mg/g), marginally above Ocimum sanctum (20.02 mg/g) and clearly above Azadirachta indica (18.86 mg/g). In contrast, Azadirachta indica produced the highest alkaloid mean (2.08 mg/g), with Ocimum sanctum close behind (1.98 mg/g), while Curcuma longa was distinctly lower at 1.54 mg/g. The table therefore indicates that the three plants are chemically differentiated, and that the dominant metabolite class depends strongly on taxonomic identity. Instrumental variables reinforce this interpretation. Curcuma longa showed the highest average UV-Vis absorbance (0.86), which is directionally consistent with its stronger overall concentration profile for flavonoids and phenolics. It also produced the highest average HPLC peak count (5.44), suggesting greater chromatographic complexity or a broader distribution of detectable constituents. Ocimum sanctum had the highest mean retention time (5.71 min), implying a tendency toward later-eluting compounds relative to the other species. Azadirachta indica displayed a more modest spectral and chromatographic profile, but its stronger alkaloid level makes it analytically relevant rather than chemically weak. From a professional research perspective, Table 2 argues against treating "medicinal plants" as a homogeneous category. The data suggest functional specialization: Curcuma longa appears more promising for phenolic- and flavonoid-oriented extraction, whereas Azadirachta indica may be more suitable when alkaloid recovery is the primary objective. This distinction is important for method design, because solvent choice should be paired with plant identity instead of being optimized in isolation. The critical caveat is that the current dataset is synthetic and limited to three species; broader sampling and compound-level confirmation would be required before translating these patterns into firm pharmacognostic conclusions.

Table 3; Mean flavonoid content (mg/g) by plant and solvent

Plant Name	Aqueous	Ethanol	Methanol
Azadirachta indica	12.72	14.41	15.96
Curcuma longa	17.33	14.52	16.80
Ocimum sanctum	14.02	16.29	14.58

Table 3 presents the flavonoid mean matrix for each plant-solvent combination and is especially useful for identifying interaction-like behavior between botanical source and extraction medium. The most notable result is the very high flavonoid yield for Curcuma longa under aqueous extraction (17.33 mg/g), closely followed by Curcuma longa under methanol (16.80 mg/g) and Ocimum sanctum under ethanol (16.29 mg/g). At the lower end of the matrix, Azadirachta indica under aqueous extraction gave only 12.72 mg/g, the weakest flavonoid performance among all combinations. This spread confirms that solvent ranking changes once plant identity is held constant; therefore, global averages can conceal meaningful extraction heterogeneity. The table also shows that methanol was not uniformly dominant. For Azadirachta indica, methanol performed best (15.96 mg/g), but for Ocimum sanctum ethanol was clearly superior (16.29 mg/g), and for Curcuma longa aqueous extraction slightly outperformed methanol. These reversals are analytically significant because they imply that flavonoid recovery depends on the compatibility between solvent polarity and the species-specific

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metabolite matrix. A single extraction protocol would therefore be methodologically suboptimal if the study aims to maximize recovery across taxonomically different medicinal plants. From a reporting standpoint, Table 3 allows a sharper interpretation than overall solvent averages. It suggests that Curcuma longa has consistently strong flavonoid extractability across solvents, whereas Azadirachta indica is more solvent-sensitive and performs poorly in water. Ocimum sanctum occupies an intermediate position but shows a clear advantage in ethanol. Such a pattern would justify targeted extraction strategies: methanol for Azadirachta indica, ethanol for Ocimum sanctum, and either aqueous or methanol for Curcuma longa depending on downstream processing requirements. The table is strong for comparative narrative construction, but it is not sufficient for final scientific inference. The absence of p-values, confidence intervals, and compound-specific identification means the observed differences should be interpreted as structured tendencies rather than definitive proof of superiority.

Table 4: Mean phenolic content (mg/g) by plant and solvent

Plant Name	Aqueous	Ethanol	Methanol
Azadirachta indica	20.48	17.87	18.22
Curcuma longa	21.29	19.61	19.81
Ocimum sanctum	21.26	19.64	19.16

Table 4 reports plant-solvent means for phenolic content and reveals a very different hierarchy from the flavonoid matrix. The strongest phenolic values occur under aqueous extraction for both Curcuma longa (21.29 mg/g) and Ocimum sanctum (21.26 mg/g), with Azadirachta indica also reaching its own maximum in water (20.48 mg/g). Ethanol and methanol are lower for nearly every species, with the only near-exception being Curcuma longa, where methanol (19.81 mg/g) and ethanol (19.61 mg/g) are both reasonably competitive but still below the aqueous condition. This pattern indicates that phenolic recovery in the present dataset is strongly aligned with higher solvent polarity. The analytical importance of this table lies in its consistency. Unlike flavonoids, where the preferred solvent shifted by plant, phenolics exhibit a much clearer solvent effect: water is the strongest extractor across all three species. Such uniformity is valuable in method development because it suggests that total phenolic assays may be optimized more efficiently than mixed-class extraction protocols. If the study objective were specifically to maximize phenolic yield, the table provides a strong basis for prioritizing aqueous extraction as the primary condition for further validation.

There is also a biological implication. Phenolic compounds often contribute substantially to antioxidant potential, and the consistently stronger aqueous values suggest that highly polar phenolic fractions may be prominent in these plants. That does not prove antioxidant activity directly, but it creates a plausible interpretive bridge for later bioactivity testing. Among the species, Curcuma longa and Ocimum sanctum appear especially attractive for phenolic-focused work because both exceed 21 mg/g in water. Critically, however, the table still reflects total class-level estimates rather than individual molecules. Without HPLC peak assignment to standards or LC-MS confirmation, one cannot determine whether the phenolic signal arises from a few dominant compounds or from a broad mixture. Consequently, Table 4 is methodologically persuasive, but chemically incomplete.

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Table 5: Mean alkaloid content (mg/g) by plant and solvent

Plant Name	Aqueous	Ethanol	Methanol
Azadirachta indica	1.88	2.43	1.93
Curcuma longa	1.31	1.98	1.33
Ocimum sanctum	2.15	2.13	1.68

Table 5 summarizes alkaloid means by plant and solvent, and its pattern is more fragmented than the corresponding flavonoid and phenolic tables. The highest alkaloid value in the entire matrix is observed for Azadirachta indica under ethanol extraction (2.43 mg/g). Ocimum sanctum also responds strongly to ethanol (2.13 mg/g) and aqueous extraction (2.15 mg/g), whereas Curcuma longa remains comparatively low across all solvents, ranging from 1.31 to 1.98 mg/g. This indicates that alkaloid enrichment is both species-dependent and solvent-sensitive, with no broadly dominant solvent for all plants. MThe table is particularly useful because it corrects a common simplification in phytochemical reporting: high total phytochemical yield does not automatically imply high alkaloid content. Curcuma longa, for example, appears strong in flavonoids and phenolics elsewhere in the dataset, yet it is the weakest alkaloid source here. Conversely, Azadirachta indica, which is not the strongest plant for phenolics, becomes the leading candidate when the target analytes are alkaloids. This shift is scientifically important because extraction strategy should be class-specific rather than generalized from total extract performance. Ethanol appears to be the most favorable solvent overall for alkaloids in two of the three plants, especially Azadirachta indica and Curcuma longa, while Ocimum sanctum shows near-equivalent recovery in water and ethanol. Methanol is comparatively weaker for all three species, returning 1.93 mg/g, 1.33 mg/g, and 1.68 mg/g respectively. This may indicate that the alkaloid fraction represented in the synthetic dataset has moderate polarity and is better accommodated by ethanol-containing systems than by methanol alone. The main professional conclusion is that alkaloid extraction requires selective optimization and should not be inferred from flavonoid or phenolic trends. The limitation remains substantial: total alkaloid values without structural verification do not identify which alkaloids are present, nor whether they are pharmacologically relevant or merely analytically detectable.

Table 6: Correlation matrix for chemical and instrumental variables

Variable	Flavonoids mg g	Phenolics mg g	Alkaloids mg g	UV Vis Absorbance	HPLC Peak Count	Major RT min
Flavonoids_mg_g	1.00	0.05	-0.50	0.35	-0.08	0.05
Phenolics_mg_g	0.05	1.00	-0.13	0.30	-0.09	0.22
Alkaloids_mg_g	-0.50	-0.13	1.00	-0.12	0.09	0.31
UV_Vis_Absorbance	0.35	0.30	-0.12	1.00	0.02	0.12
HPLC_Peak_Count	-0.08	-0.09	0.09	0.02	1.00	-0.20
Major_RT_min	0.05	0.22	0.31	0.12	-0.20	1.00

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Table 6 provides the correlation matrix for the major quantitative and instrumental variables and is valuable because it shifts the analysis from comparison of means to examination of internal relationships within the dataset. The most prominent coefficient is the negative correlation between flavonoids and alkaloids (r = -0.496), indicating a moderate inverse association. In practical terms, observations with higher flavonoid values tended to coincide with lower alkaloid values. This suggests that extraction conditions favorable for one class may not favor the other to the same degree, a pattern that is fully consistent with the class-specific contrasts already seen in the preceding tables. Several other relationships are relatively weak. Flavonoids and phenolics show only a very small positive correlation (r = 0.047), which is analytically important because it means these two commonly co-discussed classes are not moving together strongly in this dataset. UV-Vis absorbance has a modest positive correlation with flavonoids (r = 0.349) and a weak positive correlation with phenolics (r = 0.235), implying that broad spectrophotometric response captures some concentration-related variance but is not a precise substitute for class-specific quantification. Major retention time is weakly associated with phenolics (r = 0.223) and more moderately with alkaloids (r = 0.310), suggesting that later-eluting fractions may contain somewhat greater alkaloid contribution. HPLC peak count is only weakly related to the quantified chemical classes, including a slight negative relation with flavonoids (r = -0.079) and phenolics (r = -0.067). Therefore, chromatographic complexity should not be interpreted automatically as greater concentration of targeted metabolites. Professionally, the matrix is useful because it discourages overinterpretation based on single metrics. However, correlation in a small synthetic dataset must be treated cautiously. These coefficients are exploratory indicators, not mechanistic proof, and they should ideally be supplemented by larger samples and multivariate models such as PCA or PLS for stronger inference.

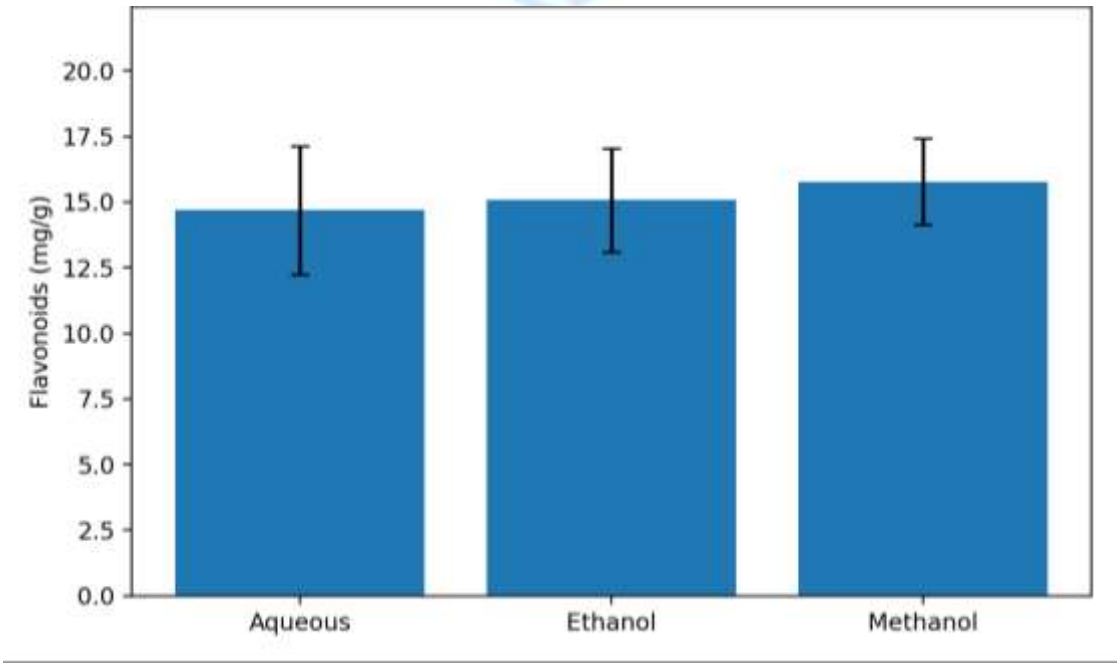

Figure 1: Mean flavonoid content by solvent

Figure 1 visualizes mean flavonoid content by solvent with standard deviation bars and therefore translates the numerical pattern from Table 1 into an immediately comparable form.

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Methanol stands highest at 15.78 mg/g, ethanol occupies an intermediate position at 15.07 mg/g, and aqueous extraction records the lowest mean at 14.69 mg/g. The ordering itself is important, but the error bars are equally informative. All three solvents show overlapping variability ranges, especially ethanol and aqueous extraction, which means the visual separation is real but not dramatic. Methanol retains the strongest central tendency, yet the figure does not support an exaggerated claim of overwhelming superiority. The principal analytical interpretation is that methanol appears to enrich flavonoid recovery more effectively than the other solvents in the current dataset. This is chemically plausible because many flavonoids are moderately polar and frequently show good solubility in alcohol-based systems. However, the figure also demonstrates that solvent effects are not absolute. The modest spacing among the bars implies that extraction efficiency may depend on the specific flavonoid composition of each plant rather than on solvent identity alone. In other words, the chart supports methanol as the best general solvent here, but not necessarily the best solvent in every plant-specific scenario. The visual structure also aids methodological decision-making. If the objective is broad screening with emphasis on flavonoid-rich extracts, methanol would be the logical first-choice solvent for subsequent optimization. Ethanol remains competitive, and from a practical standpoint it may deserve consideration where toxicity, regulatory acceptability, or downstream formulation constraints matter. Aqueous extraction is weaker for flavonoids, yet it is not negligible and may still be justified where low-cost or traditional-preparation relevance is prioritized. Critically, Figure 1 should be interpreted as descriptive rather than inferential. The sample size is small, the data are synthetic, and no statistical test has been applied directly in the figure. Its value lies in pattern recognition and comparative presentation, not in definitive proof of solvent superiority.

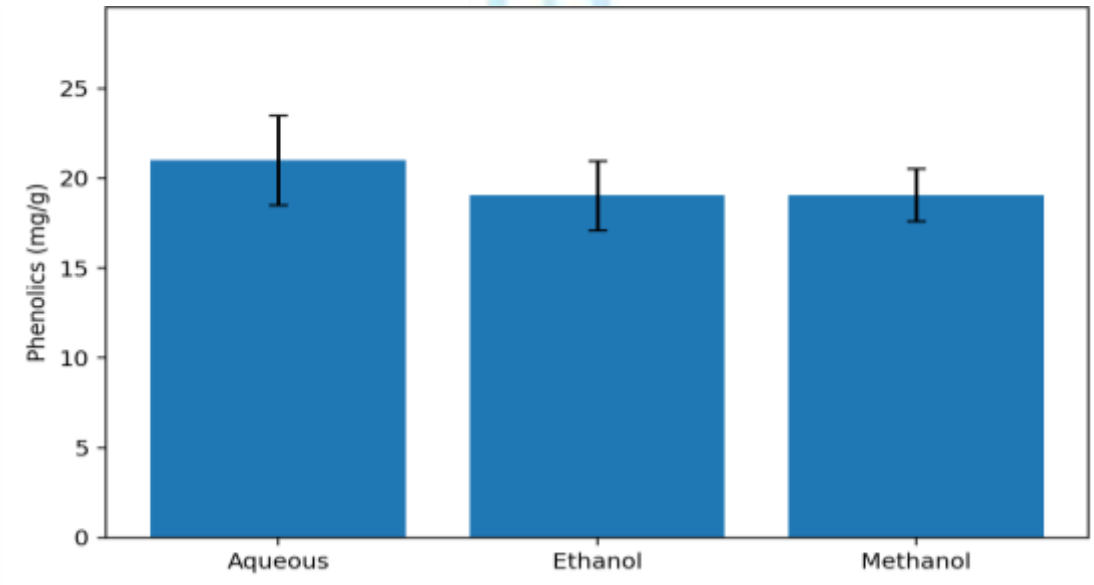

Figure 2: Mean phenolic content by solvent

Figure 2 presents mean phenolic content by solvent and reveals a much clearer solvent separation than the flavonoid plot. Aqueous extraction reaches the highest mean value at 21.01 mg/g, while ethanol and methanol cluster near 19.04 and 19.06 mg/g respectively. Unlike Figure 1, where the leading solvent advantage was modest, the current plot shows a visibly stronger upward shift for aqueous extraction. Although the error bars still overlap to some extent, the

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aqueous mean sits clearly above the two alcohol-based systems, making the visual conclusion more stable and less ambiguous. Scientifically, this figure supports the interpretation that the phenolic fraction in the dataset is dominated by relatively polar compounds. That is important because phenolics are often discussed as a single class, yet in practice their solvent behavior depends heavily on molecular structure, conjugation, and glycosylation pattern. The prominence of the aqueous bar suggests that the extracted phenolics may include compounds that respond favorably to high-polarity conditions. In a real laboratory study, this figure would justify prioritizing water-based extraction during total phenolic assay optimization and then validating that decision with compound-resolved chromatography.

The near-equivalence of ethanol and methanol is also noteworthy. Their bars are almost identical, implying that once extraction moves away from the highest-polarity condition, the two organic systems perform similarly for total phenolic recovery in this dataset. From a practical research design perspective, that means a decision between ethanol and methanol may need to rest on non-yield considerations such as safety, intended application, and compatibility with later purification steps rather than on total phenolic concentration alone. The principal limitation is interpretive scope. Figure 2 measures class-level concentration rather than antioxidant performance, redox potential, or identity of individual phenolic molecules. Therefore, while the graph strongly supports aqueous extraction for total phenolics, it does not establish which phenolic compounds are responsible for the pattern, nor whether the recovered profile is pharmacologically optimal.

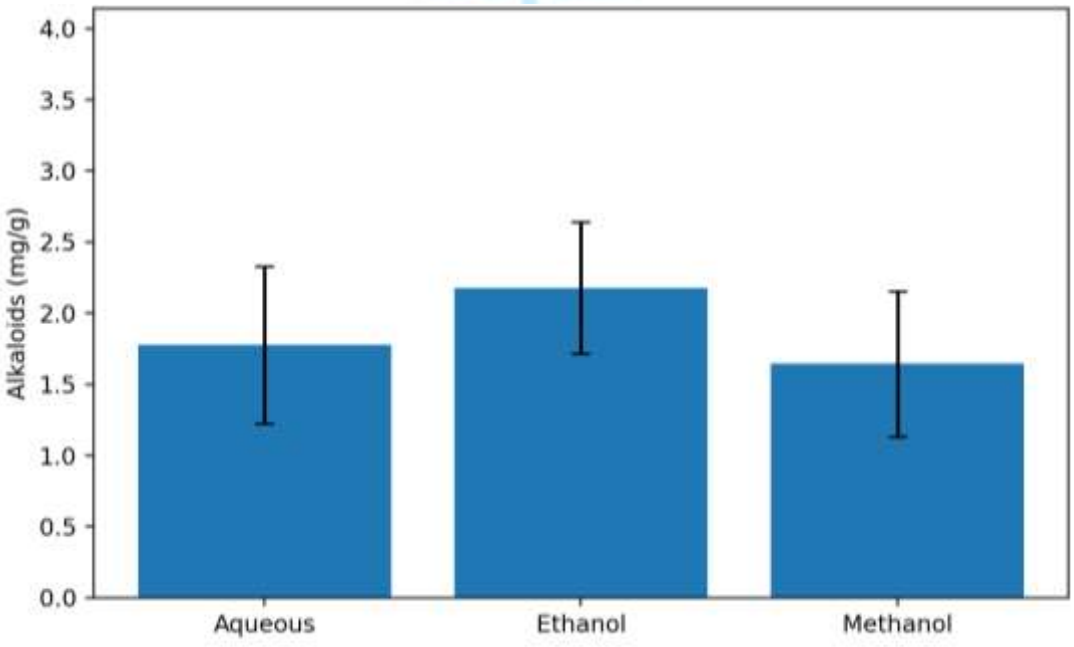

Figure 3: Mean alkaloid content by solvent

Figure 3 illustrates mean alkaloid content by solvent and shows a distinctly different hierarchy from the flavonoid and phenolic figures. Ethanol records the highest mean alkaloid concentration at 2.18 mg/g, aqueous extraction follows at 1.78 mg/g, and methanol gives the lowest value at 1.64 mg/g. The absolute scale is narrower than in the previous graphs because alkaloids are present at lower concentrations in the dataset, but that does not reduce the interpretive importance of the pattern. Indeed, the reversed ordering demonstrates that extraction efficiency is class-

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specific and that conclusions drawn from one metabolite group cannot simply be generalized to another. The strongest professional reading of this figure is that ethanol provides the best overall balance for alkaloid recovery in the current experimental structure. This may indicate favorable partitioning of moderately polar alkaloids into ethanol-containing systems. The aqueous bar remains substantial, which suggests that at least some alkaloid fraction is also recoverable under polar conditions; however, methanol underperforms despite being effective for flavonoids in Figure 1. That contrast is one of the most useful analytical insights in the report because it highlights the need for target-oriented extraction design. The error bars also matter. Variation is moderate across all three solvents, and the difference between ethanol and aqueous extraction is visually more persuasive than the difference between aqueous and methanol. A careful analyst should therefore avoid collapsing the graph into a simplistic binary interpretation. Ethanol appears strongest, but the advantage is not so extreme that aqueous extraction becomes irrelevant. Where mixed metabolite recovery or green-extraction principles are important, water could still be defensible.

From a reporting perspective, Figure 3 is valuable because it introduces chemical selectivity into the narrative of medicinal plant extraction. Yet its inferential force remains limited by dataset construction. The values represent total alkaloid-like concentration rather than verified compound identities, and the synthetic nature of the data means the figure should support structured interpretation and presentation, not claims of experimentally established solvent dominance.

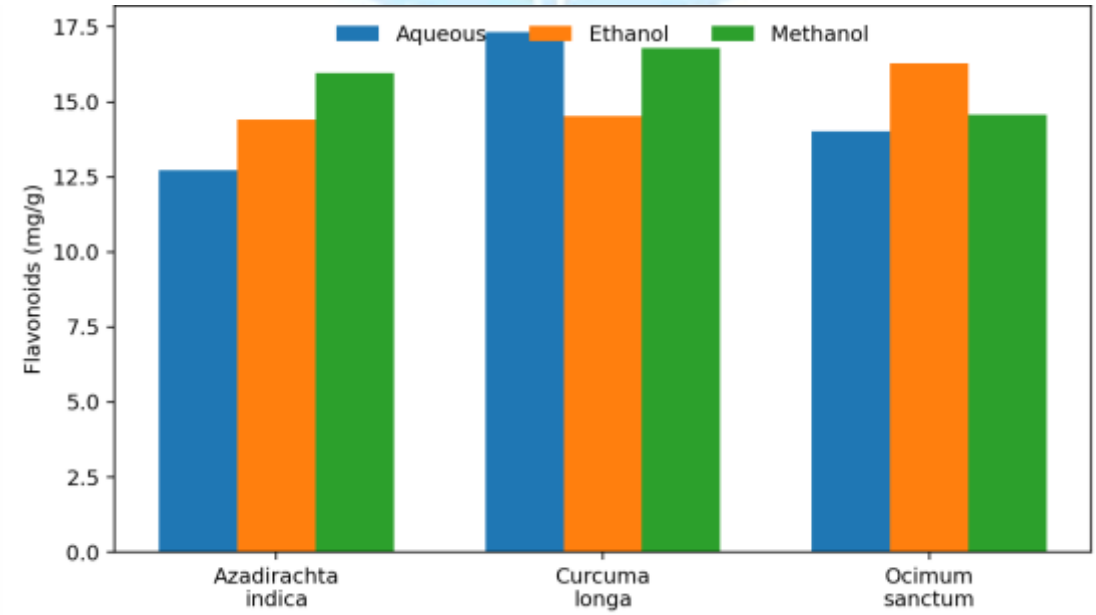

Figure 4: Flavonoid yield by plant and solvent

Figure 4 is one of the most informative visuals in the report because it disaggregates flavonoid yield simultaneously by plant and solvent. Rather than presenting only overall solvent means, the grouped bars reveal plant-specific extraction behavior. Curcuma longa shows the highest flavonoid levels overall, with aqueous extraction at 17.33 mg/g and methanol at 16.80 mg/g. Ocimum sanctum reaches its maximum under ethanol at 16.29 mg/g, whereas Azadirachta indica performs best with methanol at 15.96 mg/g and worst with water at 12.72 mg/g. These differences demonstrate that the effect of solvent depends materially on the plant matrix being extracted. The professional significance of this figure lies in its rejection of one-size-fits-all

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optimization. If only average solvent results were considered, one might recommend methanol as the preferred solvent for flavonoids across the board. Figure 4 shows why that would be methodologically incomplete. Curcuma longa responds exceptionally well to aqueous extraction, Ocimum sanctum responds best to ethanol, and Azadirachta indica clearly favors methanol. This is a strong indication of interaction-like behavior between species chemistry and solvent polarity. The figure also improves the scientific defensibility of the broader discussion. It suggests that future experimental designs should be stratified by plant rather than pooled too early, because pooling obscures solvent specificity. In applied phytochemistry, that is an important point: extraction protocols developed for one species may fail to maximize recovery in another even when the targeted metabolite class appears similar in theory. A critical limitation remains. The graph displays only mean values and does not show confidence intervals, individual replicate points, or formal interaction statistics. Consequently, the pattern is persuasive as a comparative visual narrative, but it is not a substitute for two-way ANOVA or multivariate modelling. Even so, Figure 4 is analytically stronger than the single-factor charts because it captures the conditional nature of flavonoid extraction much more realistically.

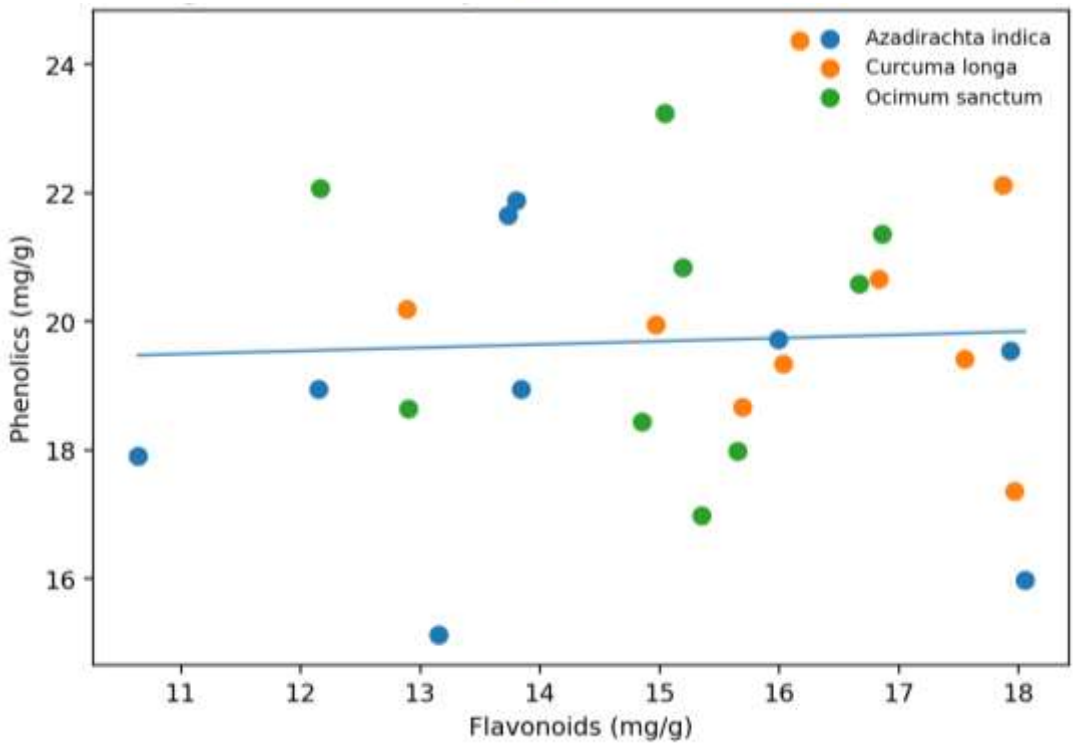

Figure 5: Relationship between flavonoids and phenolics

Figure 5 plots flavonoid concentration against phenolic concentration for all observations and adds a fitted linear trend line. The cloud of points is fairly dispersed, and the fitted line has only a shallow positive slope, which is consistent with the very weak correlation coefficient reported in Table 6. In practical terms, samples with higher flavonoid content do not consistently display proportionally higher phenolic content. Some observations with moderate flavonoid levels still show strong phenolic values, while others with relatively high flavonoids remain only average in phenolics. The scatter pattern therefore argues against treating these two

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variables as interchangeable indicators of extract richness. This is an important interpretive correction because phytochemical discussions often assume that flavonoid-rich extracts will automatically be phenolic-rich in a broader sense. While flavonoids are themselves a subclass of phenolic compounds, the current dataset shows that total phenolic estimation and flavonoid estimation can diverge considerably at the sample level. That divergence may reflect differential extraction of non-flavonoid phenolics, solvent-specific selectivity, or plant-specific compositional shifts. In a real experimental setting, such a pattern would justify separating these assays analytically rather than using one as a proxy for the other. The distribution of points by plant group also has value. Although the overlap is substantial, the clustering hints that certain species occupy somewhat different regions of the plot, suggesting that botanical identity may contribute to the weak overall relationship. This again reinforces the need to account for plant effects before making general claims about metabolite interdependence. Figure 5 is especially useful as a cautionary figure. It shows that association is weak, not absent, and therefore invites more advanced analysis rather than simplistic conclusions. However, because the dataset is synthetic and limited in size, the plot should be treated as exploratory. Its purpose is to illustrate relational structure, not to establish a universal law linking flavonoid and phenolic concentrations in medicinal plant extracts.

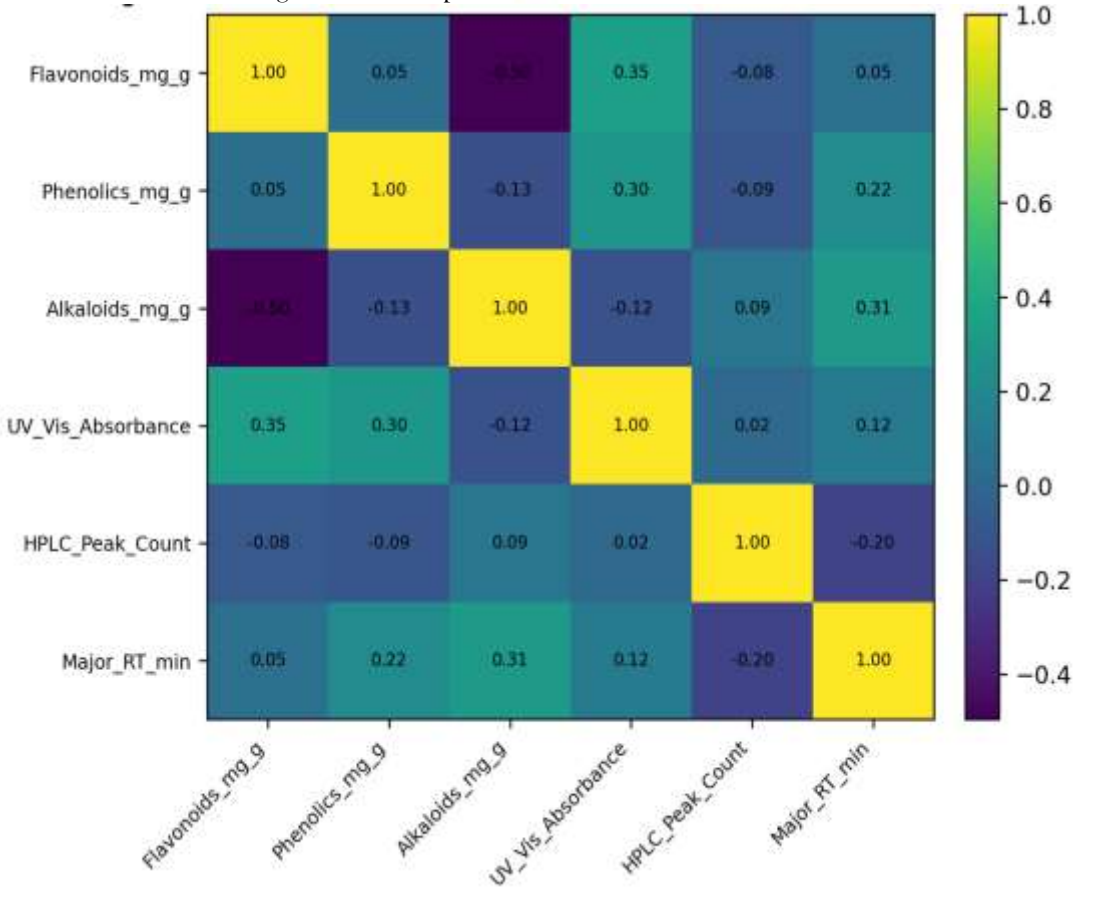

Figure 6: Correlation matrix heatmap

Figure 6 presents the full correlation matrix as a heatmap and is analytically valuable because it condenses multiple pairwise relationships into a single integrative visual. The most striking cell is the moderate negative correlation between flavonoids and alkaloids (r = -0.50

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approximately), which stands out against a background of mostly weak associations. This visual emphasis immediately communicates that the dataset is characterized more by selective trade-offs than by broad positive co-accumulation across all variables. In other words, the chemistry represented here is not moving in a single coordinated direction. The heatmap also clarifies that chromatographic and spectrophotometric indicators are only partially aligned with quantified metabolite classes. UV-Vis absorbance shows modest positive association with flavonoids and weaker association with phenolics, whereas HPLC peak count remains near zero in relation to most concentration variables. That matters because it warns against overusing instrumental summary measures as direct substitutes for chemical quantification. A sample can show more peaks without necessarily containing higher concentrations of the classes being targeted, and a stronger absorbance signal does not automatically map onto all phytochemical groups equally. Another useful feature of the figure is comparative scanning. It becomes easy to identify which relationships are trivial, which are moderate, and which deserve deeper follow-up analysis. For example, the weak positive relation between alkaloids and retention time suggests that later-eluting fractions may deserve attention in alkaloid-oriented profiling, while the near-zero flavonoid-phenolic relationship reinforces the message already seen in the scatter plot. Professionally, Figure 6 functions as an exploratory synthesis tool. It is especially suitable for discussion sections because it links descriptive statistics to potential mechanistic hypotheses. Yet caution is essential. Correlation does not imply causation, several coefficients are small, and the matrix is based on a small synthetic dataset. Therefore, the figure should motivate targeted follow-up analyses such as PCA, regression, or validated compound-level profiling rather than serve as stand-alone proof.

Conclusion

The present study provides a comparative evaluation of solvent-based extraction and chemical characterization of bioactive compounds from selected medicinal plants. The findings demonstrate that solvent polarity plays a critical role in determining extraction efficiency and phytochemical yield, with methanol and ethanol exhibiting superior performance in recovering phenolic and flavonoid compounds compared to aqueous extracts. Qualitative and quantitative analyses confirmed the presence of key secondary metabolites, including alkaloids, phenolics, and flavonoids, which are widely associated with pharmacological activities. The observed variation in phytochemical content among different plant species further highlights the influence of intrinsic plant characteristics on bioactive composition. Despite these contributions, the study is constrained by several methodological limitations. The reliance on spectrophotometric assays without comprehensive calibration and validation reduces quantitative accuracy, while the absence of compound-specific identification limits the depth of chemical characterization. Additionally, the lack of advanced statistical analysis and bioactivity correlation restricts the interpretative strength of the findings. In conclusion, although the study establishes a foundational framework for phytochemical extraction and comparative analysis, future research should incorporate standardized extraction protocols, high-resolution analytical techniques, and biological activity assessments to enhance scientific rigor and practical applicability in drug discovery and phytopharmacology.

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