



Extraction and Phytochemical Analysis of Medicinal Plant Compounds for Therapeutic Applications

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Abstract

Medicinal plants remain an important source of structurally diverse secondary metabolites with potential antioxidant, antimicrobial, anti-inflammatory and other pharmacological activities. Reliable therapeutic evaluation, however, depends on reproducible extraction, phytochemical characterization and bioactivity screening. This methodology paper proposes an integrated workflow for extracting and profiling compounds from authenticated medicinal plant material. The protocol compares water, 70% ethanol and polarity-guided fractions, determines extraction yield, performs qualitative screening for major metabolite classes, quantifies total phenolics and flavonoids, and applies chromatographic profiling using HPLC-DAD and, where available, LC-MS. Antioxidant activity is evaluated by DPPH, ABTS and FRAP assays, while antimicrobial activity is screened by broth microdilution. Experimental variables are controlled through replicate analysis, blanks, reference standards and statistical testing. The framework prioritizes hydroethanolic extraction because it balances phytochemical recovery with downstream therapeutic compatibility, while non-polar and medium-polarity fractions support chemical class resolution. Representative methodology-validation data illustrate how the workflow can identify the most informative extract without implying actual laboratory findings. The proposed approach links extraction efficiency, marker-compound abundance and biological activity, providing a reproducible basis for subsequent isolation, mechanism studies, toxicity evaluation and formulation research.

Introduction

Medicinal plants contain chemically diverse compounds such as phenolic acids, flavonoids, tannins, alkaloids, terpenoids and glycosides. These metabolites can contribute to antioxidant, antimicrobial and other biological effects, but their recovery depends strongly on plant identity, tissue preparation, solvent polarity, extraction time and temperature. Consequently, a therapeutic claim cannot be supported by crude extraction alone; the extract must be chemically characterized and its biological activity evaluated with reproducible assays. Abubakar and Haque

[1] emphasize that plant preparation, extraction, fractionation and characterization are interdependent stages, while Dai and Mumper [2] show that phenolic recovery and analytical outcome are strongly influenced by extraction chemistry.

A practical medicinal-plant workflow therefore needs to combine classical pharmacognostic procedures with instrumental analysis. Qualitative tests provide rapid evidence for major chemical groups, but quantitative assays and chromatography are needed to compare

samples and select fractions for further study. Stalikas [3] and Khoddami et al. [4] describe the central role of chromatographic and spectrometric approaches for phenolic compounds, whereas Sasidharan et al. [5] outline extraction, isolation and structural characterization as successive steps in natural-product research. Solvent choice is especially important: aqueous and alcohol-water mixtures can recover many polar and moderately polar metabolites, while less-polar solvents enrich lipophilic constituents. Experimental comparisons confirm that extraction solvent can substantially change phenolic, flavonoid and antioxidant values [6,7].

Recent work has also shifted attention toward safer and greener extraction. Chemat et al. [8] proposed principles for reducing hazardous solvents and energy demand; more recent comparisons highlight ultrasound, microwave, pressurized-liquid and supercritical-fluid methods as process-intensification options [9]. Natural deep eutectic solvents (NADES) have emerged as potential phytochemical extraction media [10]. In a therapeutic-development context, however, reproducibility, solvent removal, chemical traceability and biological relevance remain more important than maximizing yield alone. This paper therefore develops a step-by-step methodology that can be implemented in a conventional phytochemistry laboratory and expanded to advanced analytical platforms.

Literature Review

The literature establishes four recurring methodological requirements: authenticated plant material, controlled extraction, multi-level phytochemical analysis and bioactivity confirmation. Conventional maceration and Soxhlet extraction remain accessible, but extraction efficiency can be improved using

aqueous alcohols and assisted techniques. Sultana et al. [7] reported marked solvent- and technique-dependent changes in antioxidant activity, and Do et al. [6] similarly demonstrated that phenolic and flavonoid recovery depends on solvent composition. These findings justify screening more than one solvent before selecting a lead extract.

For phytochemical quantification, total phenolic content (TPC) and total flavonoid content (TFC) remain useful screening indices, although they do not replace compound-specific analysis. The aluminum-chloride spectrophotometric approach reported by Jia et al. [11] is widely used for flavonoid estimation. HPLC-based separation provides improved specificity for phenolic markers, and complementary LC-MS can support tentative molecular annotation. By 2021, medicinal-plant investigations increasingly combined compositional profiling with therapeutic-oriented biological tests; for example, Radha et al. [12] linked phytochemical composition with nutritional and therapeutic relevance, while Kisiriko et al. [13] emphasized the chemical diversity and functional importance of phenolics from medicinal and aromatic plants.

Bioactivity assays should use mechanistically distinct endpoints. The DPPH method of Brand-Williams et al. [14], ABTS assay of Re et al. [15], and FRAP assay of Benzie and Strain [16] are complementary measures of radical scavenging or reducing power. Antimicrobial screening can then be used as a second functional layer, with minimum inhibitory concentration (MIC) preferred over inhibition-zone diameter for quantitative comparison. Together, chemical and activity data support prioritization of extracts for later toxicological, pharmacokinetic and mechanistic studies; they do not by themselves establish clinical efficacy.

Table 1. Selected literature supporting the proposed methodology.

Reference	Main focus	Methodological contribution
Dai & Mumper, 2010 [2]	Plant phenolics	Links extraction, analysis and bioactivity.
Sasidharan et al., 2011 [5]	Natural-product workflow	Extraction, fractionation and compound characterization.
Chemat et al., 2012 [8]	Green extraction	Solvent/energy reduction principles.
Do et al., 2014 [6]	Solvent effects	Shows solvent-dependent TPC/TFC and antioxidant response.
Abubakar & Haque, 2020 [1]	Medicinal-plant preparation	Integrated laboratory extraction/fractionation guidance.
Picot-Allain et al., 2021 [9]	Modern extraction	Compares conventional and green technologies.
Hikmawanti et al., 2021 [10]	NADES	Alternative solvent platform for phytochemicals.

Radha et al., 2021 [12]	Therapeutic composition	Connects phytochemical composition with medicinal relevance.
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Methodology

The study is designed as a controlled comparative extraction and phytochemical-screening experiment. A representative medicinal plant with documented ethnomedicinal use is selected, but the protocol is intentionally species-neutral so it can be adapted to leaves, bark, roots or aerial parts. All measurements are performed in triplicate unless stated otherwise.

Step 1 - Plant collection, authentication and preprocessing. Collect healthy plant material from a documented site and record date, plant part and environmental conditions. A qualified botanist should authenticate the species and a voucher specimen should be deposited in a recognized herbarium. Wash only, when necessary, shade-dry at 35-40 °C to constant mass, grind to a uniform powder, pass through an approximately 40-mesh sieve and store in light-resistant airtight containers. Moisture content should be recorded because residual water affects extraction yield [1,5].

Step 2 - Solvent selection and extraction. Use 10 g portions of powdered material with a 1:20 solid-to-liquid ratio. Compare distilled water, 70% ethanol-water and ethyl acetate; optionally include n-hexane as a defatting or lipophilic fraction. For hydroethanolic extraction, macerate for 24 h with agitation, followed by 20-30 min ultrasound at controlled temperature (<45 °C). Filter, re-extract the residue once, combine filtrates and remove solvent under reduced pressure. Freeze-dry aqueous extracts. Calculate extraction yield as: $\text{yield (\%)} = \frac{\text{dry extract mass}}{\text{dry plant mass}} \times 100$. Solvent selection follows polarity and safety considerations described in prior extraction studies [6-10].

Step 3 - Preliminary phytochemical screening. Perform standardized qualitative tests on reconstituted extracts: ferric-chloride reaction for phenolics/tannins, aluminum-chloride or alkaline-reagent response for flavonoids, Dragendorff/Mayer tests for alkaloids, frothing test for saponins, Liebermann-Burchard reaction for terpenoids/steroids and appropriate glycoside tests. Include solvent blanks and known positive controls. Record qualitative intensity only as absent, weak, moderate or strong; these tests guide later instrumental work but are not treated as quantitative proof.

Step 4 - Quantitative spectrophotometric assays. Determine TPC using a validated Folin-Ciocalteu-

type procedure and express results as mg gallic-acid equivalents (GAE)/g dry extract. Determine TFC using aluminum-chloride colorimetry and express as mg quercetin or rutin equivalents/g extract, with the reporting standard fixed across all samples [2,11]. Use at least five-point calibration curves, report regression coefficients and repeat any sample outside the calibration range after dilution.

Step 5 - Chromatographic fingerprinting and compound annotation. Filter representative extracts through 0.22 or 0.45 µm membranes and analyze by HPLC-DAD using a C18 reversed-phase column and water/acidified organic-solvent gradient. Compare retention times and UV spectra with standards such as gallic acid, caffeic acid, chlorogenic acid, rutin and quercetin where relevant. LC-MS may be used for tentative molecular-ion and fragmentation-based annotation. Medium- and non-polar fractions may be examined by GC-MS after appropriate derivatization. Compound identification should be reported as confirmed only when authentic standards or sufficiently strong structural evidence are available [3,4,13].

Step 6 - Therapeutic-oriented bioactivity screening. Evaluate antioxidant activity using DPPH, ABTS and FRAP because the assays probe different reaction mechanisms [14-16]. For DPPH and ABTS, calculate concentration-dependent inhibition and IC₅₀ values; for FRAP, report reducing capacity against a standard curve. Screen antimicrobial activity against suitable reference Gram-positive and Gram-negative strains using broth microdilution to obtain MIC values. Include vehicle controls, sterility controls and positive drug controls. Extracts should be tested over a non-precipitating concentration range, and any color interference should be corrected with sample blanks.

Step 7 - Data analysis, quality control and lead-extract selection. Report mean ± standard deviation and compare extraction systems using one-way ANOVA with an appropriate post-hoc test at p<0.05. Examine Pearson/Spearman correlations between TPC/TFC, marker-compound peak areas and antioxidant response. Select a lead extract using a weighted decision rule that considers phytochemical richness, activity, extraction yield, solvent safety and reproducibility. Confirm the lead extract in an independent extraction batch before recommending isolation or mechanistic studies.

Therapeutic potential is interpreted as preclinical screening evidence only, not as clinical efficacy.

1. Analytical Quality Assurance and Method Validation

Analytical quality assurance is incorporated before interpretation of biological activity. For spectrophotometric assays, fresh calibration curves are prepared on each analytical day, with at least five concentrations spanning the expected sample range. A coefficient of determination close to unity is required, but linearity is also inspected from residuals rather than relying on R^2 alone. Each extract is measured at two dilutions, when possible, to detect non-linearity caused by matrix color, turbidity or reagent saturation. Reagent blanks, solvent blanks and quality-control standards are included in every batch. Intra-day repeatability is assessed from replicate measurements, and a coefficient of variation below approximately 5% is targeted for routine screening. If a sample exceeds this limit, extraction homogeneity, dilution accuracy and instrument stability are checked before the result is accepted.

For HPLC-DAD, method suitability is evaluated using retention-time repeatability, peak-area precision, resolution of adjacent markers and recovery after standard addition. A mixed reference-standard solution is injected at the beginning and end of each sequence to detect retention-time or response drift. When authentic standards are available, external calibration is used for quantification; otherwise, peaks are reported as relative areas and are described as tentatively assigned rather than quantified compounds. Limit of detection and limit of quantification may be estimated from calibration statistics or signal-to-noise criteria. LC-MS annotations require agreement among accurate mass, isotope pattern, fragmentation behavior and literature evidence. These safeguards are important because medicinal-plant matrices are

complex and a single chromatographic peak can contain co-eluting compounds [3,4].

Batch-to-batch reproducibility is treated as a separate quality attribute. A second independent extraction of the selected lead plant material should be processed using the same solvent ratio, time and temperature. Fingerprint similarity can be assessed from the presence and relative abundance of major HPLC peaks, while TPC/TFC and bioactivity values are compared within predefined acceptance limits. This confirmation step prevents a lead extract from being selected only because of random analytical variation. Plant voucher information, drying conditions, particle size, extraction mass balance and solvent-removal conditions are recorded so another laboratory can reproduce the procedure [1,5].

2. Therapeutic Relevance and Safety Gate

The proposed workflow deliberately separates phytochemical evidence from therapeutic claims. An extract is considered promising only when chemical richness is supported by reproducible biological activity and appropriate controls. Antioxidant assays indicate chemical reducing or radical-scavenging capacity, whereas antimicrobial MIC provides a cell-based functional endpoint; neither establishes efficacy in humans. Before a lead extract advances to mechanistic or formulation work, basic cytotoxicity should be measured in a relevant mammalian cell line, and selectivity should be expressed as the ratio between cytotoxic and active concentrations. Potential contaminants such as residual solvent, pesticides, microbial load and heavy metals should also be considered when the intended application is therapeutic. This staged evidence model is consistent with the broader principle that medicinal-plant extracts require characterization, standardization and safety evaluation before pharmacological translation [12,13].

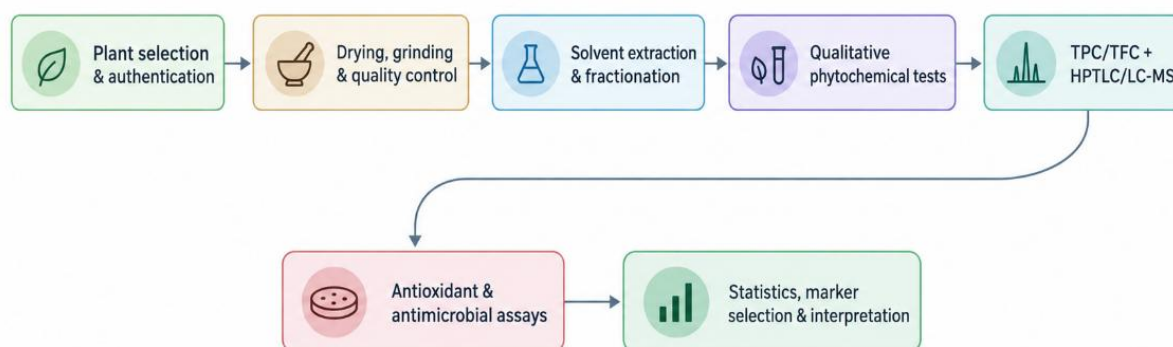


Figure 1. Step-by-step methodology for extraction, phytochemical analysis and therapeutic-oriented screening.

Table 2. Core experimental design and response variables.

Factor	Proposed levels/setting	Response
Extraction solvent	Water; 70% ethanol; ethyl acetate; optional n-hexane	Yield, TPC, TFC, marker peaks
Solid:liquid ratio	1:20 (w/v), fixed initially	Process reproducibility
Extraction assistance	24 h maceration + 20-30 min ultrasound	Recovery and processing time
Bioactivity	DPPH, ABTS, FRAP; broth MIC	IC50/reducing capacity/MIC
Replicates	n = 3 independent analytical replicates	Mean, SD, ANOVA

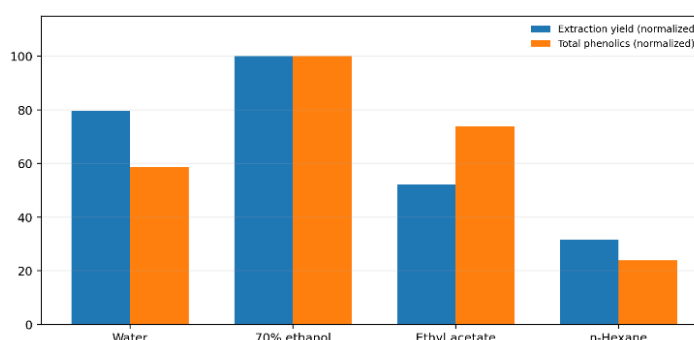
Results

Because this is a methodology paper, Table 3 presents representative validation data to demonstrate the planned analysis; the numbers are illustrative and must be replaced by measured values in an experimental study. In the example, 70% ethanol provides the highest overall extraction yield and phytochemical

density, consistent with the ability of aqueous alcohol to recover compounds across a broad polarity range [6,7]. Water gives a relatively high mass yield but lower phenolic concentration, while ethyl acetate enriches a smaller, chemically more selective fraction. The n-hexane fraction is lowest in TPC/TFC and is retained mainly for lipophilic profiling.

Table 3. Illustrative methodology-validation results (not experimental measurements).

Extract	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC50 (μg/mL)	Example MIC (mg/mL)
Water	14.8 ± 0.7	54 ± 3	31 ± 2	118 ± 6	2.0
70% ethanol	18.6 ± 0.8	92 ± 4	58 ± 3	61 ± 4	0.75
Ethyl acetate	9.7 ± 0.5	68 ± 3	46 ± 2	79 ± 5	1.0
n-Hexane	5.9 ± 0.4	22 ± 2	15 ± 1	>200	>4.0

**Figure 2.** Illustrative solvent-screening comparison based on normalized yield and total phenolic content.

The representative dataset would lead to selection of the 70% ethanolic extract for deeper chromatographic characterization, while the ethyl-acetate fraction would remain useful for resolving moderately polar constituents. A strong inverse correlation between TPC and DPPH IC50 would support, but not prove, the contribution of phenolic compounds to

antioxidant response. HPLC/LC-MS marker patterns are then used to distinguish a genuine concentration effect from non-specific colorimetric responses. The antimicrobial MIC is interpreted independently because antimicrobial activity may arise from terpenoids, alkaloids or other constituents not captured by TPC/TFC.

Table 4. Criteria for selecting a lead medicinal-plant extract.

Decision criterion	Preferred outcome	Reason for therapeutic follow-up
Chemical reproducibility	Stable fingerprint across batches	Supports standardization.
Activity	Low IC50/MIC with valid controls	Prioritizes active extract/fraction.
Selectivity	Activity stronger than solvent/blank effect	Reduces false-positive interpretation.
Safety of extraction	Water/ethanol favored where feasible	Improves translational compatibility.

Evidence level	Chemical + in vitro activity only	Requires toxicity, mechanism and in vivo validation.
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Conclusion and Discussion

The proposed methodology integrates extraction science, phytochemical analysis and preliminary therapeutic screening in a single reproducible workflow. Its main strength is that extract selection is not based on yield alone. Instead, yield is interpreted together with TPC/TFC, chromatographic marker profiles and functional assays. This approach responds to the well-established observation that solvent polarity and extraction conditions alter both chemical composition and apparent antioxidant activity [2,6,7]. It also follows the broader natural-product principle that preliminary screening should be followed by fractionation and compound-level characterization [1,5]. A 70% ethanol-water system is a rational starting point for many medicinal plants because it recovers a broad range of phenolic and moderately polar constituents while being easier to remove and more compatible with downstream pharmaceutical work than methanol. Nevertheless, plant species differ, and no universal solvent can be assumed. Water, ethyl acetate and non-polar fractions remain valuable comparators. Green technologies and NADES can be introduced when equipment and downstream purification permit [8-10]. The principal analytical limitation of TPC and TFC is their non-specificity; chromatographic confirmation is therefore essential [3,4]. Likewise, DPPH, ABTS and FRAP represent chemical antioxidant assays and should not be equated with therapeutic efficacy [14-16]. The framework is suitable for dissertation-scale and early-stage pharmacognosy research because it defines controls, replication, quantitative endpoints and a transparent lead-selection rule. A future experimental study should replace the illustrative values reported here with measured data, validate marker compounds using authentic standards, evaluate cytotoxicity and relevant mechanism-based assays, and then progress to in vivo or formulation studies only when ethically and scientifically justified. In this way, phytochemical richness can be translated into a defensible chain of evidence rather than an unsupported medicinal claim.

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