



Extraction and Phytochemical Analysis of Bioactive Compounds from Medicinal Plants for Pharmaceutical Applications

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Abstract

Medicinal plants remain important sources of chemically diverse molecules for drug discovery, standardized herbal products and pharmaceutical excipients. The present methodology establishes an integrated procedure for extracting and characterizing bioactive constituents from selected medicinal plants, with emphasis on reproducibility, solvent efficiency and analytical confirmation. Three representative medicinal plants—*Azadirachta indica*, *Ocimum tenuiflorum* and *Moringa oleifera*—are proposed as comparative matrices. Authenticated plant material is washed, shade-dried, milled and extracted with 70% ethanol using ultrasound-assisted extraction, while aqueous and absolute-ethanol extractions are retained as solvent controls. Crude extracts are concentrated under reduced pressure and evaluated by preliminary tests for alkaloids, phenolics, flavonoids, tannins, saponins and terpenoids. Total phenolic and flavonoid contents are quantified spectrophotometrically, antioxidant capacity is assessed by DPPH and ABTS assays, and chemical fingerprints are generated by thin-layer chromatography, HPLC-DAD and FTIR spectroscopy. Extraction yield, phytochemical content and antioxidant response are compared statistically to identify the most promising bioactive-rich extract for further pharmaceutical development. The illustrative validation dataset suggests that hydroethanolic extraction can provide a favorable compromise between mass yield, phenolic recovery and radical-scavenging activity. The proposed workflow links botanical authentication, extraction, screening, quantification and instrumental characterization in a single methodology suitable for subsequent formulation, bioactivity-guided fractionation and quality-control studies.

Introduction

Plant-derived natural products have historically contributed to pharmacotherapy and continue to provide structurally diverse lead molecules, standardized extracts and chemical scaffolds for modern drug discovery. The usefulness of a medicinal plant, however, is not determined solely by its ethnomedicinal reputation. A reproducible pharmaceutical investigation requires correct botanical identification, controlled sample preparation, an extraction

procedure compatible with the chemistry of target metabolites, and analytical methods capable of establishing composition and biological relevance. Sasidharan et al. (2011) emphasized that extraction, isolation and characterization form a continuous analytical sequence, while Altemimi et al. (2017) showed that phytochemical investigations increasingly combine classical solvent extraction with

chromatographic and spectroscopic identification.

Extraction is especially critical because plant tissues contain compounds with widely different polarity, molecular weight, stability and cellular localization. Phenolics, flavonoids, alkaloids, terpenoids, tannins and saponins therefore respond differently to solvent polarity, temperature, extraction time and solid-to-liquid ratio. Azmir et al. (2013) and Azwanida (2015) described maceration and Soxhlet extraction as practical conventional techniques but noted the advantages of ultrasound-, microwave- and other assisted methods in reducing extraction time and solvent consumption. Experimental evidence also shows that solvent choice can substantially alter both phytochemical yield and measured antioxidant activity (Sultana et al., 2009; Nguyen et al., 2016).

For pharmaceutical applications, crude extraction should be followed by chemical standardization rather than reliance on a single qualitative test. Phenolic compounds are routinely quantified because of their association with antioxidant and other biological activities (Dai & Mumper, 2010), while chromatographic fingerprinting helps distinguish complex extracts and supports quality control. Accordingly, this paper proposes a stepwise methodology that integrates extraction yield, preliminary phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), radical-scavenging activity, TLC, HPLC-DAD and FTIR. The central objective is to identify a reproducible bioactive-rich extract that can proceed to fractionation, formulation and pharmacological evaluation without overstating preliminary phytochemical data as proof of therapeutic efficacy.

Literature Review

The literature consistently identifies extraction as the first major determinant of phytochemical recovery. Naczak and Shahidi (2006) reviewed the occurrence, extraction and analysis of phenolic compounds and explained that solvent polarity and interactions between phenolics and matrix components govern solubility. Sultana et al. (2009) experimentally compared solvents and extraction techniques for medicinal plants and showed that antioxidant activity varies with the extraction system. Dai and Mumper (2010) further summarized extraction, purification and analysis of plant phenolics, linking careful sample preparation to reliable antioxidant and anticancer investigations. These findings justify the use of a mixed aqueous-organic solvent for

broad recovery of polar and moderately polar constituents.

Method-development studies support optimization of time, temperature and extraction technology rather than adoption of a single universal procedure. Vuong et al. (2013) demonstrated that extraction temperature, time and solvent-to-leaf ratio significantly affected phenolic recovery and antioxidant activity from *Carica papaya* leaves. Azmir et al. (2013) reviewed conventional and non-conventional extraction technologies and stressed that plant matrix, solvent, temperature, pressure and time jointly control efficiency. Azwanida (2015) similarly concluded that the choice of extraction technique must consider both the target phytochemicals and the limitations of the plant material. Nguyen et al. (2016), working with *Phyllanthus amarus*, found strong effects of both solvent and extraction method and reported that assisted extraction could improve recovery while reducing energy requirements.

Analytical screening is strengthened when broad phytochemical assays are coupled with quantitative and activity-based measurements. The Folin-Ciocalteu method remains widely used for estimating total phenolics (Singleton et al., 1999), while the colorimetric flavonoid procedure described by Zhishen et al. (1999) provides a practical estimate of flavonoid abundance. Antioxidant potential can be compared using the DPPH radical method of Brand-Williams et al. (1995) and the ABTS radical-cation assay of Re et al. (1999). Earlier work on standardized medicinal-plant extracts also demonstrated that polyphenol-rich preparations may exhibit substantial antioxidant activity (Pietta et al., 1998). Collectively, these studies support an integrated methodology in which extraction efficiency, phytochemical concentration, chemical fingerprinting and functional antioxidant response are interpreted together rather than independently.

Methodology

Study design. A comparative laboratory methodology is proposed for three commonly studied medicinal plants: *Azadirachta indica* (neem) leaves, *Ocimum tenuiflorum* (holy basil) leaves and *Moringa oleifera* leaves. The plants are used as representative matrices rather than as claims of therapeutic superiority. Each sample is processed in triplicate. The principal extraction system is 70% ethanol because a hydroethanolic solvent can recover a broad range of polar and moderately polar constituents; distilled water and absolute

ethanol are included as controls. The design follows the extraction principles summarized by

Azmir et al. (2013), Azwanida (2015) and Altemimi et al. (2017).

Table 1. Standardized extraction conditions used in the proposed methodology.

Factor	Proposed condition	Purpose
Plant material	Authenticated, mature leaves; n = 3 independent batches	Reduce botanical and batch variability
Drying and milling	Shade-dry at 35–40 °C; grind and sieve to ≤0.5 mm	Limit thermal degradation and improve solvent contact
Main solvent	70% ethanol in water	Broad recovery of phenolics and moderately polar metabolites
Solid:liquid ratio	1:20 (w/v)	Provide adequate mass transfer without excessive solvent
Assisted extraction	Ultrasound, 30 min, 35–40 °C	Shorten extraction time and improve cell disruption
Replicates	Three analytical replicates per extract	Support mean, SD and inferential statistics

Step 1: Collection, Authentication and Pre-treatment

Fresh, disease-free plant material is collected and authenticated by a qualified botanist or herbarium. Voucher specimens are deposited for traceability. The material is rinsed rapidly with distilled water, blotted dry and shade-dried at 35–40 °C until constant mass. Dried material is ground, passed through a 0.5 mm sieve and stored in amber airtight containers at low humidity until extraction. Controlled drying and particle-size reduction are important because excessive heat may degrade phenolics, while smaller particles improve solvent contact (Dai & Mumper, 2010; Azwanida, 2015).

Step 2: Solvent Extraction and Concentration

Ten grams of powdered sample is mixed with 200 mL of 70% ethanol. The suspension is

sonicated for 30 min at 35–40 °C, cooled and filtered through Whatman No. 1 paper. The plant residue is re-extracted once under the same conditions, and the filtrates are combined. Parallel extractions with distilled water and absolute ethanol are performed to examine the effect of solvent polarity. The combined extracts are concentrated below 45 °C using a rotary evaporator; aqueous portions may be freeze-dried. Dried extracts are weighed and stored at 4 °C in amber vials. Extraction yield is calculated as: Yield (%) = [mass of dried extract / mass of dry plant powder] × 100. This comparative solvent approach is consistent with reports that solvent and assisted-extraction conditions strongly influence phytochemical recovery (Sultana et al., 2009; Vuong et al., 2013; Nguyen et al., 2016).



Figure 1. Stepwise workflow for extraction and phytochemical characterization of medicinal-plant bioactives.

Step 3: Preliminary Phytochemical Screening

Each dried extract is reconstituted at 10 mg/mL. Standard qualitative reactions are used to screen major phytochemical groups: ferric chloride reaction for phenolics/tannins, alkaline-reagent

or aluminum-chloride reaction for flavonoids, Dragendorff or Mayer reaction for alkaloids, frothing test for saponins and Salkowski reaction for terpenoids/steroids. Results are recorded using a semi-quantitative scale (–, +, ++, +++) under identical extract concentration and

reagent volumes. These tests are treated as preliminary evidence only and are followed by chromatographic confirmation, as recommended for plant-extract characterization (Sasidharan et al., 2011; Altemimi et al., 2017).

Step 4: Quantitative Phenolic and Flavonoid Estimation

TPC is measured by the Folin-Ciocalteu method using gallic acid for the calibration curve and is reported as mg gallic acid equivalents (GAE) per g dried extract (Singleton et al., 1999). TFC is estimated colorimetrically using aluminum chloride with quercetin or rutin as the reference standard and is expressed as mg quercetin equivalents (QE) per g extract, following the principles of Zhishen et al. (1999). All measurements are performed in triplicate against appropriate reagent blanks. Because Folin-Ciocalteu response is not absolutely specific for phenols, TPC is interpreted as a comparative reducing-capacity index and is supported by HPLC-DAD fingerprinting (Naczki & Shahidi, 2006).

Step 5: Antioxidant and Instrumental Characterization

DPPH radical scavenging is assessed at a series of extract concentrations and percentage inhibition is calculated from the decrease in absorbance; IC₅₀ is obtained by nonlinear or linearized concentration-response fitting (Brand-Williams et al., 1995). ABTS radical-cation decolorization is performed as a complementary antioxidant assay because it accommodates both hydrophilic and lipophilic antioxidants (Re et al., 1999). TLC fingerprints are first generated on silica gel plates using a solvent system selected after pilot trials and visualized under UV light and with suitable derivatization reagent. For HPLC-DAD, a C18 reversed-phase column is used with acidified water and acetonitrile/methanol gradient elution, monitoring representative wavelengths near 280, 320 and 360 nm for phenolic subclasses. FTIR spectra from 4000–400 cm⁻¹ are recorded to identify broad

functional-group patterns. Instrumental fingerprints are used for comparative standardization rather than definitive structural identification unless reference standards or MS/NMR confirmation are available (Sasidharan et al., 2011; Altemimi et al., 2017).

Step 6: Statistical Analysis and Pharmaceutical Lead Selection

Results are reported as mean ± standard deviation. Extraction systems are compared by one-way or two-way ANOVA, as appropriate, followed by Tukey post-hoc testing at $p < 0.05$. Pearson correlation may be used to relate TPC/TFC with DPPH or ABTS response. The lead extract is selected using a composite interpretation of extraction yield, phenolic/flavonoid content, antioxidant activity, chemical fingerprint quality and practical solvent safety. This avoids choosing an extract solely because it gives the largest crude mass and aligns the methodology with the need for standardized, chemically characterized material before pharmaceutical bioassays.

Results

Because no primary laboratory dataset was supplied, the numerical values in this section are an illustrative methodology-validation dataset designed to show how results should be reported and interpreted; they are not presented as experimentally measured values. In the illustrative comparison, the 70% ethanolic extracts produced the most balanced performance across extraction yield, phenolic recovery and antioxidant response. Absolute ethanol yielded less total extract, whereas water produced moderate mass yield but lower flavonoid recovery in two of the three representative plants. This pattern is chemically plausible because hydroethanolic mixtures improve penetration of hydrated plant tissues while retaining capacity for moderately polar compounds (Azmir et al., 2013; Nguyen et al., 2016).

Table 2. Illustrative quantitative results for the proposed 70% ethanolic extracts (mean ± SD, n = 3).

Plant / 70% ethanol extract	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC ₅₀ (μg/mL)
<i>Azadirachta indica</i>	18.6 ± 0.8	112.4 ± 4.5	58.7 ± 2.8	42.6 ± 2.1
<i>Ocimum tenuiflorum</i>	20.9 ± 0.7	138.5 ± 5.2	71.3 ± 3.1	31.8 ± 1.6
<i>Moringa oleifera</i>	22.4 ± 0.9	126.7 ± 4.8	64.1 ± 2.6	36.5 ± 1.9

Within the illustrative dataset, *O. tenuiflorum* showed the highest TPC and TFC and the lowest DPPH IC₅₀, indicating the strongest radical-scavenging response. *M. oleifera* produced the

highest crude extraction yield, but its lower phenolic density relative to *O. tenuiflorum* illustrates why yield alone is an insufficient basis for pharmaceutical lead selection. A negative

relationship between TPC and DPPH IC₅₀ would be expected because stronger antioxidant extracts require lower concentrations to produce 50% radical inhibition. Such interpretation is

consistent with the broader evidence that plant phenolics contribute substantially to measured antioxidant activity (Pietta et al., 1998; Dai & Mumper, 2010).

Table 3. Illustrative qualitative phytochemical profile (– absent; + low; ++ moderate; +++ strong reaction).

Phytochemical class	A. indica	O. tenuiflorum	M. oleifera	Analytical follow-up
Phenolics / tannins	+++	+++	+++	TPC, HPLC-DAD
Flavonoids	++	+++	+++	TFC, TLC/HPLC
Alkaloids	++	+	+	TLC; targeted LC if required
Saponins	++	+	++	Fractionation / confirmatory assay
Terpenoids	++	+++	++	TLC, FTIR; GC-MS for volatile fraction

TLC and HPLC-DAD would be expected to reveal multiple chemical zones or peaks rather than a single dominant compound, reflecting the complexity of medicinal-plant extracts. The presence of reproducible retention patterns across independent batches would support use of the chromatogram as a quality-control fingerprint. FTIR bands associated with O–H stretching, aromatic C=C vibrations, carbonyl functions and C–O linkages would provide complementary evidence for polyphenolic and related functional groups but would not by themselves identify individual molecules. The lead extract should therefore advance to bioactivity-guided fractionation, reference-standard HPLC/LC-MS analysis, toxicity screening and pharmaceutical formulation studies rather than being considered a finished therapeutic product.

Conclusion and Discussion

The proposed methodology provides a practical and defensible route for converting medicinal-plant material into analytically characterized extracts suitable for pharmaceutical research. Its principal strength is the integration of controlled botanical preparation, comparative solvent extraction, qualitative screening, quantitative TPC/TFC assays, antioxidant testing and chromatographic/spectroscopic fingerprinting. Literature up to 2017 shows that extraction outcome is highly dependent on solvent polarity, temperature, time, matrix characteristics and extraction technology; therefore, standardization of these variables is essential for reproducibility (Azmir et al., 2013; Azwanida, 2015; Altemimi et al., 2017). Hydroethanolic ultrasound-assisted extraction is proposed as the main procedure because it combines broad phytochemical recovery with relatively mild

operating conditions and manageable solvent removal. For pharmaceutical application, the results must be interpreted conservatively. Qualitative color tests and antioxidant assays are useful for screening but do not establish clinical efficacy, compound identity or safety. The strongest extract should instead be treated as a lead material for further fractionation, LC-MS/NMR identification, mechanism-based bioassays, cytotoxicity assessment, stability testing and formulation development. Batch-to-batch chromatographic fingerprints should also be incorporated into quality-control specifications. Thus, the methodology supports a rational progression from traditional medicinal-plant use toward reproducible chemical evidence and pharmaceutical development while maintaining clear separation between preliminary phytochemical activity and validated therapeutic claims.

References

- Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. <https://doi.org/10.3390/plants6040042>
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>

- Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4, 196. <https://doi.org/10.4172/2167-0412.1000196>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352. <https://doi.org/10.3390/molecules15107313>
- Naczek, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 41(5), 1523–1542. <https://doi.org/10.1016/j.jpba.2006.04.002>
- Nguyen, V. T., Pham, H. N. T., Bowyer, M. C., van Altena, I. A., & Scarlett, C. J. (2016). Influence of solvents and novel extraction methods on bioactive compounds and antioxidant capacity of *Phyllanthus amarus*. *Chemical Papers*, 70(5), 556–566. <https://doi.org/10.1515/chempap-2015-0240>
- Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *Journal of Agricultural and Food Chemistry*, 46(11), 4487–4490. <https://doi.org/10.1021/jf980310p>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1), 1–10. <https://doi.org/10.4314/ajtcam.v8i1.60483>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14(6), 2167–2180. <https://doi.org/10.3390/molecules14062167>
- Vuong, Q. V., Hirun, S., Roach, P. D., Bowyer, M. C., Phillips, P. A., & Scarlett, C. J. (2013). Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica papaya* leaf aqueous extracts. *Journal of Herbal Medicine*, 3(3), 104–111. <https://doi.org/10.1016/j.hermed.2013.04.004>
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)