



Medicinal plants as sources of natural antioxidants: Phytochemical complexity, extraction optimization and methodological challenges

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DOI: <https://doi.org/10.66856/ijfsn.2026.11.4.11185>

Abstract

Medicinal plants contain chemically diverse mixtures of phenolic acids, flavonoids, tannins, terpenoids, alkaloids, vitamins and other metabolites that may participate in redox reactions. Their antioxidant potential has attracted interest in pharmacognosy, food science, agriculture and natural-product research. However, the measured activity of a plant extract is not an intrinsic property independent of method: it depends on species identity, genotype, plant organ, developmental stage, environmental conditions, drying and storage, solvent composition, extraction energy and time, assay chemistry, and the way results are expressed. This review critically examines the relationship between phytochemical complexity, extraction design and antioxidant assessment. Conventional solvent extraction is compared with ultrasound-assisted, microwave-assisted, pressurized-liquid and supercritical-fluid approaches, with attention to yield, selectivity, thermal sensitivity, solvent burden and scale-up. Common assays—including DPPH, ABTS, FRAP, CUPRAC and ORAC—are discussed in terms of reaction mechanisms, analytical strengths and limitations. Total phenolic and flavonoid assays are treated as operational measurements rather than compound-specific determinations. Chromatographic and spectrometric tools, especially HPLC-DAD and LC-MS/MS, are considered necessary for chemical characterization and interpretation of complex extracts. A central conclusion is that no single extraction method or antioxidant assay can provide a complete ranking of medicinal plants. More reliable studies combine validated chemical profiling, complementary antioxidant assays, transparent reporting, appropriate controls and, where relevant, cell-based or *in vivo* evidence. The review proposes a practical framework for improving reproducibility and avoiding unsupported translation of *in vitro* antioxidant results into therapeutic claims.

Keywords: Medicinal plants natural antioxidants, phenolic compounds, extraction optimization, green extraction, DPPH, ABTS, FRAP, ORAC, chromatographic profiling

Introduction

Medicinal plants are chemically dynamic biological systems rather than uniform sources of a single active ingredient. Their extracts may contain hundreds of primary and secondary metabolites, including phenolic acids, flavonoids, anthocyanins, tannins, lignans, terpenoids, carotenoids and ascorbate. Some of these compounds can donate electrons or hydrogen atoms, chelate transition metals, or influence oxidation-related pathways under particular chemical or biological conditions. Such properties help explain the continued use of plant materials as sources of antioxidants for food preservation, nutraceutical development and natural-product discovery (Zhang *et al.*, 2022; Shi *et al.*, 2022)^[13, 15].

Nevertheless, the term antioxidant is often used too broadly. A compound that reduces a synthetic radical in a test tube is not necessarily absorbed, stable, bioavailable or effective in a living organism. Chemical assays measure defined reactions under controlled conditions; they do not by themselves establish prevention or treatment of disease. Reviews of antioxidant methodology have repeatedly highlighted differences in reaction chemistry, solvent effects, kinetics and reporting conventions, all of which complicate comparison between studies (Schaich *et al.*, 2015; Munteanu & Apetrei, 2021)^[7, 12].

The apparent antioxidant potential of a medicinal plant is shaped by a chain of interdependent decisions. Plant authentication and sample handling influence the material

entering the laboratory. Extraction conditions determine which molecules are recovered and whether they remain chemically intact. Analytical procedures then measure either broad response classes or individual compounds, while antioxidant assays capture only selected reactions. A result reported as a single number therefore compresses a much more complex chemical and methodological history.

This review synthesizes evidence on three connected questions: how phytochemical diversity influences antioxidant measurements; how extraction methods can be selected and optimized without compromising compound integrity or sustainability; and how antioxidant capacity should be assessed and reported to improve reproducibility. Rather than treating high *in vitro* activity as proof of therapeutic efficacy, the review emphasizes analytical validity, appropriate interpretation and research designs that connect chemical composition with biological relevance.

Review approach and scope

This is a critical narrative review of literature concerning medicinal-plant antioxidants, phenolic extraction, analytical characterization and antioxidant assay limitations. The discussion draws on methodological reviews and research articles available through scholarly publishers and open-access repositories, including work on plant-derived phenolics, green extraction technologies and chemical or cell-based antioxidant assays. The aim is conceptual synthesis rather than a systematic estimate of effect size. No

new experiments or pooled quantitative analyses were conducted. A formal systematic review would require a prespecified search strategy, reproducible database searches, screening records and an explicit risk-of-bias assessment; these elements should be added if the target journal requires a systematic-review format.

Phytochemical complexity and antioxidant behaviour

1. Phenolic acids, flavonoids and tannins

Phenolic compounds are among the most frequently investigated plant antioxidants because their aromatic structures and hydroxyl substituents can support electron transfer, hydrogen donation and stabilization of the resulting radicals. Phenolic acids such as caffeic, ferulic, gallic and p-coumaric acids differ in hydroxylation, methoxylation and conjugation, producing differences in redox potential, solubility and reaction kinetics. Flavonoids—including flavonols, flavones, flavanones, flavan-3-ols and anthocyanins—also vary in the number and position of hydroxyl groups, the presence of a 2,3-double bond, and the degree of glycosylation. These structural features can influence activity, but rankings depend on the assay system and reaction conditions (Zhang *et al.*, 2022; Shi *et al.*, 2022) [13, 15].

Tannins comprise hydrolysable and condensed forms with multiple phenolic groups. Their strong reducing and protein-binding properties can contribute to assay responses, yet they may also complicate extraction and analysis through interactions with proteins, polysaccharides and other macromolecules. A crude extract containing a high concentration of tannins may show strong *in vitro* activity while having limited suitability for a particular formulation because of astringency, instability or poor absorption. Accordingly, a high total phenolic response should be treated as a screening indicator, not as a complete assessment of quality or biological value.

2. Non-phenolic compounds and matrix effects

Antioxidant-related responses are not restricted to phenolics. Ascorbic acid, tocopherols, carotenoids and certain sulfur-containing or nitrogen-containing metabolites can contribute directly or indirectly to redox behaviour. Their recovery differs with polarity, volatility, sensitivity to oxygen and heat, and the plant matrix. A hydroalcoholic extraction may recover polar phenolics effectively but under-represent lipophilic carotenoids or tocopherols. Conversely, non-polar solvents may recover lipophilic constituents while excluding many glycosylated phenolics. Consequently, the antioxidant activity of an extract reflects the selected extraction window rather than the full metabolome of the plant.

Plant organs also differ chemically. Leaves, flowers, roots, bark, seeds and fruits can contain distinct metabolite profiles, while maturity, season, soil conditions, water availability, light exposure, herbivory and genotype may alter the abundance of individual compounds. Post-harvest delay, drying temperature, moisture content, light exposure and storage can further modify the profile. These sources of variation should be controlled or recorded before attributing differences in antioxidant results to species-level properties.

3. Synergy, antagonism and the limits of crude-extract interpretation

Mixtures may behave differently from isolated compounds because constituents can regenerate one another, compete

for reaction partners, alter solubility or interfere analytically. A crude extract can therefore show additive, synergistic or antagonistic responses depending on concentration, matrix and assay chemistry. Demonstrating synergy requires a defined comparison against an appropriate additive or dose-response model; a crude extract outperforming one isolated compound at a single concentration is not sufficient evidence. Bioassay-guided fractionation, chemical profiling and reconstitution experiments can help determine whether activity is associated with particular molecules or combinations.

Extraction methods: recovery, selectivity and sustainability

1. Conventional solvent extraction

Maceration, percolation, infusion, decoction and Soxhlet extraction remain common because they are accessible and can be adapted to different plant materials. Solvent polarity, solvent-to-solid ratio, particle size, temperature, extraction time and agitation influence mass transfer and the balance between recovery and degradation. Water and aqueous ethanol are often relevant where food-compatible or relatively low-toxicity solvents are desired. Methanol may be useful in analytical research but is unsuitable for many food or consumer applications unless removed and controlled according to applicable requirements. Solvent choice should follow the intended compounds and use case rather than convention alone.

Long extraction times or high temperatures may increase measured yield while also promoting oxidation, hydrolysis or transformation of sensitive compounds. A higher crude-extract yield does not necessarily mean a higher concentration of target antioxidants: the additional mass may consist of sugars, organic acids, pigments, salts or other co-extractives. Studies should therefore report both extract yield and a meaningful chemical or activity metric, ideally with a clear denominator such as milligrams of target compound per gram of dry plant material.

2. Ultrasound-assisted extraction

Ultrasound-assisted extraction uses acoustic cavitation to disrupt plant structures and improve solvent penetration and mass transfer. It can reduce extraction time and solvent consumption relative to some conventional procedures. Its performance depends on frequency, power, probe or bath configuration, temperature control, solvent, solid-to-liquid ratio and matrix properties. Excessive sonication can heat samples or promote oxidation, while differences between ultrasonic devices can limit reproducibility. Reports should specify the equipment configuration and actual operating conditions rather than stating only that ultrasound was used (Cannavacciuolo *et al.*, 2024) [4].

3. Microwave-assisted extraction

Microwave-assisted extraction uses dielectric heating to accelerate heating of solvent and plant moisture, potentially improving cell disruption and compound release. It can be rapid and efficient, but local overheating and pressure development may affect thermolabile constituents. The suitability of a protocol depends on solvent dielectric properties, vessel design, sample moisture, temperature, power and time. Scale-up requires particular care because heating patterns in larger systems may differ from those in

laboratory vessels. Yield and antioxidant response should be assessed alongside marker-compound stability.

4. Pressurized-liquid and supercritical-fluid extraction

Pressurized-liquid extraction uses elevated temperature and pressure to maintain solvent in the liquid state and enhance diffusion and solubility. It may reduce extraction time but requires equipment designed for pressure and careful control of thermal exposure. Supercritical carbon dioxide is particularly useful for lipophilic constituents and can reduce residual organic-solvent concerns; however, its low polarity limits recovery of many polar phenolics unless modifiers such as ethanol are introduced. Capital costs, operational complexity and the need to match solvent properties to the target compounds can constrain adoption.

5. Green extraction and responsible solvent selection

Green extraction is not defined solely by the use of a novel instrument. A credible assessment considers solvent toxicity, renewability, energy demand, extraction time, solvent recovery, waste generation, process safety and the quality of the resulting extract. Ethanol-water mixtures, water-based methods and selected bio-based solvents may reduce environmental burden, but the best option depends on the target chemistry and intended application. Natural deep eutectic solvents and other alternative systems are promising, although viscosity, downstream recovery, toxicity evaluation and analytical compatibility remain practical concerns. Recent reviews emphasize that green technologies should be assessed against clear performance and sustainability criteria rather than assumed to be superior in every case (Cannavacciuolo *et al.*, 2024^[4]; Palos-Hernández & González-Paramás, 2025)^[8].

Extraction optimization: from trial-and-error to experimental design

Extraction is a multivariable process. Solvent composition, temperature, time, solid-to-liquid ratio, particle size and extraction energy may interact, so changing one factor at a time can miss important interactions and produce a local rather than global optimum. Response surface methodology (RSM), factorial designs and other design-of-experiments approaches allow several variables to be evaluated systematically. The response should not be limited to total phenolic content or a single radical-scavenging assay. A more defensible optimization may use multiple responses, including extract yield, concentration of selected marker compounds, antioxidant activity, solvent use and energy demand.

Optimization objectives can conflict. Conditions that maximize crude yield may dilute target compounds, while conditions that maximize a DPPH response may favour a narrow group of compounds or introduce matrix interference. Desirability functions or multi-criteria decision methods can be used where the objective is explicitly defined. The selected optimum should be verified with independent replicate extractions, and the predicted and observed responses should be reported. Validation should include variability estimates and, where possible, a comparison with a conventional baseline method.

At minimum, an extraction study should identify the plant taxon and voucher specimen where feasible; plant organ and collection conditions; drying and milling procedures; moisture basis; solvent identity and purity; solvent

composition; solid-to-liquid ratio; temperature; time; extraction cycles; equipment details; filtration and concentration steps; storage conditions; extract yield; and the basis used for reporting activity. These details are essential for reproducibility and for comparing studies conducted in different laboratories.

Analytical assessment of phytochemical composition

1. Total phenolic and total flavonoid assays

The Folin–Ciocalteu method is frequently used to estimate total phenolic content, often expressed as gallic acid equivalents. However, the reagent responds to reducing substances beyond phenolics, including some sugars, ascorbate and other matrix constituents. The result is therefore an operational measure of reducing capacity under the assay conditions rather than a direct sum of all phenolic molecules. Aluminium-complexation methods for total flavonoids also show structure-dependent responses; different flavonoid subclasses do not necessarily produce equivalent signals. Such methods are useful for screening and batch comparison when standardized, but should not replace compound-specific analysis.

2. Chromatographic and spectrometric profiling

HPLC coupled with diode-array detection can separate and quantify selected compounds when suitable standards and validated conditions are available. LC-MS/MS provides additional selectivity through mass-to-charge information and fragmentation patterns, while high-resolution mass spectrometry can support broader metabolite annotation. GC-MS is useful for volatile or derivatized constituents but is not a universal method for non-volatile phenolics. NMR can support structural elucidation and quantitative profiling in suitable settings. Untargeted metabolomics can reveal patterns and candidate markers, but tentative annotations should not be presented as confirmed identifications without adequate evidence.

Reliable chemical characterization requires calibration, selectivity, linearity, limits of detection and quantification where relevant, recovery, precision, matrix-effect assessment and stability testing. Reference standards should be used for definitive quantification when available. Results from different platforms are not automatically comparable because extraction, ionization, detector response and data-processing workflows differ. A useful strategy combines broad screening with targeted quantification of representative marker compounds, then evaluates whether the markers plausibly explain the measured antioxidant response (Shi *et al.*, 2022; Zhang *et al.*, 2022)^[13, 15].

Antioxidant assays: what they measure and what they miss

1. DPPH and ABTS radical-scavenging assays

DPPH is a stable synthetic radical commonly monitored by a change in absorbance after reaction with an extract. ABTS uses the ABTS radical cation and can be applied to a wider range of hydrophilic and lipophilic sample types depending on solvent conditions. Both are convenient for screening, but responses depend on solvent, radical concentration, reaction time, pH, steric accessibility and the kinetics of individual compounds. Pigmented extracts may interfere with absorbance readings, and slow-reacting antioxidants can be underestimated if readings are taken too early. Results from DPPH and ABTS are method-specific and

should not be treated as interchangeable measures of a universal antioxidant capacity (Schaich *et al.*, 2015; Munteanu & Apetrei, 2021)^[7, 12].

2. FRAP and CUPRAC

FRAP estimates the ability of sample constituents to reduce a ferric complex under acidic conditions. CUPRAC measures reduction of a copper (II) complex under its defined reaction conditions. These electron-transfer assays are relatively straightforward and useful for comparison within a consistent protocol. They do not directly measure every mechanism by which antioxidants may act, and reaction pH can alter the response of particular compounds. Compounds that react slowly or have limited solubility in the assay medium may be under-represented.

3. ORAC and other hydrogen-atom-transfer approaches

ORAC follows the protection of a fluorescent probe against oxidation by peroxy radicals and incorporates reaction kinetics into the measurement. It can provide complementary information to electron-transfer assays, but it is sensitive to

probe behaviour, temperature, radical generation, instrument settings and data-analysis choices. Other approaches, including lipid-oxidation models and cellular oxidative-stress assays, address different aspects of antioxidant behaviour. The selected assay should match the question being asked rather than be chosen solely because it is familiar or inexpensive.

4. Why multiple assays are necessary

No single chemical assay captures all antioxidant mechanisms, compartments or biological conditions. DPPH, ABTS, FRAP, CUPRAC and ORAC can rank the same extracts differently because they use different reagents, reaction media and endpoints. Assays also differ in their sensitivity to hydrophilic and lipophilic compounds. A well-designed study therefore selects at least two complementary methods based on distinct chemical principles, reports full conditions and controls, and avoids combining unlike outputs into a single unvalidated score. Assay-specific units—such as Trolox equivalents or Fe(II) equivalents—must be retained and clearly defined. There is no universal conversion factor between methods (Schaich *et al.*, 2015; Munteanu & Apetrei, 2021)^[7, 12].

Table 1: Common methods used to characterize plant antioxidants

Method	Main information	Strengths	Important limitations
Folin–Ciocalteu	Overall reducing response often reported as total phenolics	Simple and useful for screening	Not specific to phenolics; matrix reducers interfere
Aluminium-complexation flavonoid assay	Operational estimate of selected flavonoid responses	Accessible and low cost	Response varies among flavonoid structures
DPPH	Reaction with a stable synthetic radical	Rapid, inexpensive and widely used	Solvent, kinetics, colour and steric effects
ABTS	Reaction with ABTS radical cation	Adaptable to varied solvent systems	Protocol-dependent; colour and timing effects
FRAP	Ferric reducing power under acidic conditions	Straightforward electron-transfer assay	Does not represent all antioxidant mechanisms
CUPRAC	Reduction of a copper (II) complex	Useful complementary reducing-capacity measure	Dependent on pH, reaction time and matrix
ORAC	Protection of a fluorescent probe against peroxy radicals	Kinetic information; complementary mechanism	Instrument-sensitive and method-dependent
HPLC-DAD / LC-MS	Separation and characterization of individual metabolites	Chemical specificity and marker analysis	Standards, validation and instrument access required
Cell-based assays	Cellular response under defined exposure conditions	Adds biological context beyond cell-free chemistry	Cell model, dose, cytotoxicity and uptake affect interpretation

From chemical activity to biological relevance

Cell-free assays are valuable for initial screening, but their relationship to biological effects is indirect. In living systems, plant compounds may be poorly absorbed, rapidly metabolized, bound to proteins, transformed by gut microorganisms or present at concentrations below those used in chemical assays. Antioxidant-related effects may also arise through modulation of endogenous defence systems, inflammatory signalling or enzyme activity rather than direct radical scavenging. These possibilities require separate experimental evidence and should not be inferred from DPPH or FRAP results alone (Munteanu & Apetrei, 2021)^[7].

Cell-based experiments can test whether an extract changes oxidative-stress endpoint in a biological context, but they introduce their own challenges. Cytotoxicity, cell permeability, exposure duration, solvent controls, extract colour or fluorescence, and the relevance of the cell model must be addressed. A reduction in a fluorescent oxidation signal may reflect interference with the probe or loss of

viable cells rather than a protective effect. Positive controls, vehicle controls, concentration-response curves and independent replication are essential.

Animal or human studies are needed to address absorption, distribution, metabolism, excretion, safety and outcomes in complex organisms. Such work is resource-intensive and must follow relevant ethical and regulatory requirements. Claims about prevention or treatment of disease require evidence appropriate to the claim; chemical antioxidant activity alone is not sufficient. This distinction is especially important when translating traditional medicinal use into commercial health claims.

Methodological challenges and reporting priorities

Three recurrent problems limit the interpretability of medicinal-plant antioxidant research. First, plant materials are often inadequately characterized, making it difficult to distinguish biological variation from methodological variation. Second, extraction procedures and assay conditions are incompletely reported, preventing replication.

Third, results are sometimes interpreted as proof of health benefit despite being based only on chemical assays. These problems can be reduced through taxonomic authentication, detailed sample metadata, validated analytical procedures, transparent reporting and restrained interpretation.

At the experimental-design stage, researchers should define the intended use of the extract and the chemical classes of interest. Extraction should be optimized against more than one endpoint where possible. Assay blanks should account for extract colour and turbidity; calibration and quality-control samples should be included; replicate extractions should be distinguished from repeat instrument readings; and uncertainty should be reported. Results should state whether they are normalized to dry plant mass, extract mass or another basis. Reporting only percentage inhibition at one concentration provides limited information and can obscure differences in dose-response behaviour.

Interlaboratory comparability will require harmonized reporting of extraction conditions, reference standards, reaction times, temperature, pH, reagent concentrations, wavelengths, calibration ranges and calculation procedures. Openly sharing protocols, raw absorbance or chromatographic data where possible, and analysis scripts would make results easier to verify. Standardization should not imply that one assay is universally correct; rather, methods should be selected and reported in ways that allow their strengths and limitations to be evaluated.

Research gaps and future directions

Future studies should move from undirected screening toward chemically informed research questions. One priority is to link extract composition with activity using targeted quantification, metabolomics and multivariate analysis while guarding against overfitting and correlation-based causal claims. Another is to compare conventional and green extraction methods using equivalent plant batches, consistent analytical endpoints and transparent resource accounting. Process evaluation should include solvent recovery, energy use, waste generation and scalability in addition to yield.

A second priority is improved assay design. Combining complementary cell-free methods with lipid-oxidation models or cell-based assays can reveal whether the observed activity persists in more complex systems. Kinetic data, concentration-response curves and mechanistic controls can help distinguish rapid reactions from slow or secondary effects. Where cellular systems are used, studies should evaluate cytotoxicity and potential analytical interference. Better integration of chemical and biological evidence will reduce the risk of overstating the meaning of an *in vitro* antioxidant result.

Medicinal-plant research also needs stronger attention to reproducibility across geography, season and cultivation conditions. Reference materials, voucher specimens, genetic authentication where appropriate and chemical fingerprints can help identify sources of variation. Research on understudied regional flora may be valuable, but it should include sustainable collection practices, conservation considerations and appropriate documentation of traditional knowledge. The objective should be not merely to identify plants with high assay values, but to establish reproducible composition, credible activity and a realistic pathway toward safe use.

Conclusion

Medicinal plants remain important sources of chemically diverse compounds with measurable antioxidant-related

activity. Yet the activity observed in an extract is shaped by plant identity, growing conditions, processing, extraction selectivity and assay chemistry. Conventional and emerging extraction technologies each offer benefits and constraints; optimization should balance chemical recovery, stability, safety, resource use and intended application. Broad spectrophotometric assays are useful screening tools but cannot substitute for compound-specific characterization or biological validation. DPPH, ABTS, FRAP, CUPRAC and ORAC provide complementary, method-specific information and should not be treated as interchangeable measures of a universal antioxidant property. Progress in this field depends on transparent methods, validated chemical analysis, complementary assays, appropriate controls and cautious interpretation. A rigorous connection between phytochemical composition, reproducible activity and biological relevance is more valuable than a simple ranking of medicinal plants by a single antioxidant score.

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