



Phytochemistry and antioxidant potential of *Ocimum tenuiflorum* L.: A critical review of bioactive compounds, extraction methods and analytical approaches

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Abstract

Ocimum tenuiflorum L. (syn. *Ocimum sanctum* L.), commonly known as holy basil or tulsi, is an aromatic medicinal plant widely used in traditional systems of medicine across South Asia. Its biological properties have been associated with a chemically diverse group of constituents, including phenolic acids, flavonoids, terpenoids, essential-oil components and other specialized metabolites. This review critically discusses the phytochemical composition of *Ocimum tenuiflorum*, factors affecting extraction and analytical recovery, and commonly applied methods for evaluating antioxidant activity. Particular attention is given to the relationship between plant material, solvent polarity, extraction conditions, chemical profiling and assay selection. *In vitro* assays such as DPPH, ABTS, ferric-reducing antioxidant power (FRAP) and oxygen-radical absorbance capacity (ORAC) provide complementary rather than interchangeable estimates of antioxidant behaviour. Results reported across studies are difficult to compare when plant provenance, chemotype, processing, extraction yield, concentration units and assay protocols are not adequately described. The review identifies recurring methodological limitations, including overreliance on a single antioxidant assay, incomplete reporting of extract composition, and the tendency to infer biological efficacy from chemical assays alone. Improved standardization, chromatographic characterization, appropriate controls and carefully designed biological studies are needed to establish reproducible relationships between phytochemical composition and antioxidant effects. The evidence supports *Ocimum sanctum* L. as a promising source of bioactive compounds, while also indicating that claims of therapeutic effectiveness require evidence beyond *in vitro* antioxidant measurements.

Keywords: *Ocimum tenuiflorum*, *Ocimum sanctum*, tulsi, phytochemistry, phenolics, flavonoids, essential oil, antioxidant activity, extraction, DPPH, FRAP

Introduction

Medicinal plants remain important sources of compounds used in traditional healthcare and as starting points for pharmacological investigation. Their chemical diversity reflects interactions among genotype, environment, development and ecological pressures. *Ocimum tenuiflorum* L., commonly referred to as tulsi or holy basil, belongs to the family Lamiaceae and is cultivated and naturalized in many tropical and subtropical regions. The name *O. sanctum* L. is widely encountered in older literature and remains common in pharmacognosy and ethnomedicinal publications; authors should state the accepted taxonomic name and relevant synonym consistently.

Tulsi has a prominent place in Indian traditional medicine and household practice. Leaves and aerial parts are used in preparations intended for a variety of complaints, and the plant is also valued as an aromatic herb. Traditional use provides a rationale for scientific investigation, but it does not by itself establish clinical efficacy or safety for a particular indication. A central research question is how variation in the plant's chemical composition relates to the biological activities reported for extracts and isolated constituents.

Antioxidant research is one of the most frequently used approaches for characterizing tulsi extracts. Plant-derived phenolics can donate electrons or hydrogen atoms, chelate certain metal ions, and influence oxidation-related reactions under defined experimental conditions. However, antioxidant capacity measured in a chemical assay is not equivalent to antioxidant action in a living organism. Absorption, metabolism, distribution, concentration at the target site and interactions with biological systems all affect

in vivo outcomes. This review therefore considers phytochemical and analytical evidence together and highlights the limitations that must be addressed when comparing published results.

Review approach and scope

This narrative review is organized around the phytochemical groups reported for *O. tenuiflorum*, extraction and sample-preparation variables, analytical approaches used to characterize constituents, and assays applied to estimate antioxidant activity. It synthesizes established concepts in natural-products chemistry and antioxidant methodology rather than presenting a new systematic meta-analysis. For a journal submission, the authors should document the final database search, date range, search strings, screening procedure and inclusion/exclusion criteria, and should verify each cited reference against the original publication.

Relevant literature can be identified using combinations of the terms "*Ocimum tenuiflorum*", "*Ocimum sanctum*", "holy basil", "tulsi", "phytochemistry", "phenolic compounds", "essential oil", "antioxidant", "DPPH", "ABTS", "FRAP" and "chromatography". Studies should be assessed for botanical authentication, plant part, geographic origin, extraction details, analytical validation, replication and appropriate controls. When quantitative results are compared, differences in units and assay conditions must be resolved before interpreting relative potency.

Botanical identity and sources of chemical variation

Ocimum sanctum L. is an aromatic, branched herb with opposite leaves and characteristic inflorescences. Its morphology, leaf colour, aroma and growth habit can vary

among cultivated forms. Botanical authentication is essential because related *Ocimum* species may be traded under overlapping common names and may have substantially different essential-oil profiles. Voucher specimens deposited in a recognized herbarium provide a reproducible record of plant identity.

The composition of a medicinal plant is not fixed. Genotype or chemotype, soil properties, temperature, rainfall, light exposure, season, plant age, developmental stage and plant organ may all affect metabolite accumulation. Post-harvest factors—including delay before drying, drying temperature, particle size, storage duration, oxygen exposure and packaging—can further change the chemical profile. Studies that omit these variables risk attributing variation in antioxidant results to biological differences when it may arise from processing or analytical procedures.

Major phytochemical groups

1. Phenolic acids and related compounds

Phenolic acids and related polyphenols are important targets in tulsi phytochemical research. Rosmarinic acid is frequently discussed in Lamiaceae plants, while caffeic-acid-related compounds and other phenolics have also been investigated in *Ocimum* materials. Their hydroxyl groups can participate in redox reactions, although the magnitude of activity depends on molecular structure, assay environment and concentration. The presence and abundance of an individual phenolic should be established by suitable chromatographic methods and, where possible, comparison with authentic standards rather than inferred solely from a colourimetric total-phenolic assay.

2. Flavonoids

Flavonoids are a structurally diverse group of plant polyphenols that may contribute to measured reducing capacity and radical-scavenging activity. Differences in hydroxylation, glycosylation and other structural features influence solubility and reaction kinetics. Total-flavonoid measurements are useful as broad screening indicators but are not compound-specific. Reporting a total value as equivalents of a reference standard does not mean that the extract contains that standard compound.

3. Essential oils and terpenoids

The essential oil of *Ocimum sanctum* contains volatile constituents whose relative proportions vary among plant material and chemotypes. Eugenol, methyl eugenol, β -caryophyllene, and other terpenoid or phenylpropanoid constituents have been reported in different *Ocimum* oils, but no single profile should be assumed to represent all tulsi samples. Hydrodistillation conditions, plant freshness, storage and gas-chromatographic method can influence the profile. Volatile-oil composition should therefore be reported for the actual sample under study.

4. Other constituents

The broader chemical profile may include triterpenoids, sterols, fatty constituents, pigments and other metabolites. These groups can contribute to the overall properties of crude extracts, although their abundance depends strongly on solvent and processing. A crude extract is a complex mixture; observed activity cannot normally be assigned to one compound without fractionation, compound-level characterization and follow-up testing.

Extraction methods and factors affecting recovery

Extraction determines which constituents enter the test sample and at what relative abundance. Water, ethanol, methanol, aqueous alcohol mixtures and less-polar solvents are used according to the target compounds and intended application. Water and aqueous ethanol are relevant to many traditional or food-oriented preparations, while analytical studies may use methanol or other solvents to improve recovery of particular metabolites. Solvent selection should consider chemical polarity, safety, intended use, downstream analysis and environmental impact.

1. Conventional extraction

Maceration and infusion are operationally simple but can require extended contact time and may provide variable extraction efficiency. Soxhlet extraction maintains repeated contact with heated solvent and can improve recovery for suitable compounds, but prolonged heating may degrade thermolabile constituents. Hydrodistillation is used to obtain volatile oils and should not be treated as equivalent to extraction of non-volatile phenolics. The method should be selected according to the chemical fraction of interest.

2. Assisted extraction technologies

Ultrasound-assisted extraction can improve solvent penetration and mass transfer, while microwave-assisted extraction may reduce extraction time by promoting rapid heating. These approaches can be efficient, but performance depends on solvent composition, temperature, power, time and solid-to-liquid ratio. Excessive energy input or heat may degrade sensitive compounds. Comparisons should be based on extraction yield and chemical recovery, not only on the antioxidant activity of a single concentration.

3. Minimum reporting requirements

A reproducible extraction protocol should state the authenticated plant part, collection location and season, fresh or dried status, drying conditions, particle size, sample mass, solvent identity and concentration, solid-to-liquid ratio, extraction temperature and duration, number of extraction cycles, filtration and concentration procedures, final extract yield, storage conditions and the basis used to express concentration. Residual solvent and solvent blanks should be considered where relevant. Extract yield and activity per unit mass of extract answer different questions and should not be confused.

Analytical characterization

Preliminary screening tests can indicate broad classes of constituents, but they are insufficient for definitive identification. High-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC), often coupled with diode-array detection or mass spectrometry, are useful for profiling non-volatile constituents. Gas chromatography–mass spectrometry (GC–MS) is commonly used for volatile-oil analysis. Compound identification is strengthened by retention behaviour, mass spectral evidence, authentic standards and orthogonal methods where available.

Total phenolic content is often estimated using the Folin–Ciocalteu method. The reagent responds to reducing substances and is not exclusively specific to phenolics; results are best described as assay-defined total reducing capacity expressed as equivalents of the calibration

standard. Total flavonoid assays likewise depend on reagent chemistry and the structural classes present. Validation should include calibration range, linearity, precision, blank correction, recovery and detection or quantification limits where quantitative claims are made.

Chemical fingerprints can support quality control and comparisons between batches. However, fingerprint similarity does not establish equivalent biological activity, and a high total-phenolic value does not identify the compounds responsible. A robust workflow combines broad screening with targeted quantification of relevant markers and, where justified, untargeted profiling.

Antioxidant assays and interpretation

1. DPPH radical-scavenging assay

The DPPH assay estimates the ability of a test sample to reduce a stable coloured radical under specified conditions. A decrease in absorbance is commonly used to calculate percentage scavenging relative to a control. Results depend on solvent, incubation time, wavelength, sample colour and reaction kinetics. Coloured or turbid extracts require appropriate sample blanks. IC₅₀ values should only be compared when experimental conditions and calculation procedures are sufficiently similar.

2. ABTS radical-cation assay

The ABTS assay uses a coloured radical cation and can accommodate a broader range of sample polarities than some other assays, depending on the protocol. As with DPPH, measured activity is influenced by reaction time, solvent and concentration. Reporting the radical-generation procedure, dilution, calibration standard and calculation method is necessary for reproducibility.

3. Ferric-reducing antioxidant power (FRAP)

FRAP estimates the capacity of a sample to reduce ferric to ferrous iron under acidic assay conditions. It provides a measure of reducing power rather than a direct measure of radical scavenging in a biological system. The response may differ from DPPH or ABTS because the assays involve different reaction mechanisms and chemical environments.

4. ORAC and complementary methods

ORAC evaluates inhibition of oxidation initiated by peroxy radicals and commonly uses a fluorescent probe. Other approaches, including metal-chelation assays and cellular oxidative-stress models, may provide additional information. No single assay captures all mechanisms associated with antioxidant behaviour. Using multiple methods with different reaction principles can offer a more balanced characterization.

5. Essential controls and data reporting

Antioxidant experiments should include reagent blanks, solvent controls, a suitable positive control, independent replicates and a clearly defined concentration series. The basis of concentration—such as mass of dried extract per volume—must be stated. Results should include measures of variability and an appropriate statistical analysis. A dose-response curve is preferable to a single concentration. Chemical assays should be described as *in vitro* antioxidant-capacity tests, not as proof that an extract prevents disease or produces clinical benefit.

Interpreting relationships between phytochemistry and antioxidant activity

A plausible relationship exists between some phenolic constituents and *in vitro* antioxidant responses, but correlation does not demonstrate causation. Extracts contain multiple compounds that can interact additively, synergistically or antagonistically, and some assay signals may arise from non-phenolic reducing agents. A high total-phenolic estimate may coincide with strong activity in one assay but not another because the assays differ in reaction mechanism and solvent environment.

To test constituent–activity relationships, researchers can combine validated chromatographic quantification with concentration-response assays, fractionation and targeted testing of isolated compounds. Multivariate analysis may help explore patterns across well-characterized samples, but sample size, model assumptions, multiple testing and independent validation must be considered. Mechanistic conclusions should be proportional to the evidence available.

Safety, biological relevance and translational limitations

The use of tulsi in traditional practice should not be interpreted as a guarantee that every extract, dose or preparation is safe. Safety depends on composition, route, exposure, dose, duration and individual circumstances. Essential oils and concentrated extracts can differ substantially from customary food-like preparations. Claims about therapeutic benefits should be supported by appropriate toxicological, pharmacokinetic and clinical evidence, and potential interactions or contraindications should be evaluated by qualified professionals.

Chemical antioxidant assays are screening tools. They do not reproduce absorption, metabolism, tissue distribution or endogenous antioxidant systems. Some compounds can behave differently in biological settings than in cell-free assays, and excessive concentrations may have effects unlike those observed at physiologically achievable levels. Translational research should proceed through validated chemical characterization, relevant cellular models, suitable toxicity evaluation and appropriately designed human studies where warranted.

Methodological gaps and future research priorities

A recurring limitation in the literature is incomplete reporting of botanical identity, extraction conditions and chemical composition. Studies also vary in reference standards, assay protocols and units, which complicates comparison. Future work would benefit from authenticated voucher specimens, harmonized extraction protocols, reporting of extract yield, validated marker quantification and at least two complementary antioxidant assays. Raw data, calibration details, sample blanks and statistical methods should be reported transparently.

Research priorities include investigating seasonal and geographical variation, defining chemotypes using reproducible analytical fingerprints, and determining which constituents explain activity across assay systems. Green extraction methods deserve evaluation where they reduce solvent use or energy demand without compromising reproducibility. Biological relevance should be examined using carefully selected cellular or organismal models, with positive and negative controls and dose ranges justified by exposure considerations. Studies intended to support

therapeutic claims require safety assessment and clinical evidence rather than extrapolation from chemical assays.

Conclusion

Ocimum tenuiflorum L. is a chemically diverse medicinal and aromatic plant whose phenolic compounds, flavonoids, volatile constituents and other metabolites make it a useful subject for natural-products research. Its measured phytochemical composition and antioxidant activity are shaped by plant identity, environmental conditions, sample handling, extraction method and assay design. DPPH, ABTS, FRAP and ORAC assays provide complementary information and should be interpreted according to their underlying chemistry. Stronger evidence will come from standardized plant material, transparent extraction reporting, validated chemical characterization, multiple complementary assays and appropriately designed biological studies. The plant's traditional importance and promising laboratory findings justify continued investigation, while claims of clinical efficacy must remain proportionate to the available evidence.

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