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Antioxidant activity and total phenolic content of medicinal plant leaf extracts

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Abstract

Oxidative stress the imbalance between Reactive Oxygen Species (ROS) generation and cellular antioxidant defences lies at the mechanistic core of a growing list of chronic diseases including cardiovascular disease, type 2 diabetes, neurodegenerative conditions, and several cancers. Dietary and therapeutic antioxidants can tip this balance by scavenging free radicals, chelating pro-oxidant metals, and upregulating endogenous antioxidant enzymes. Medicinal plants remain among the richest and most structurally diverse natural sources of antioxidant phytochemicals, with phenolic acids, flavonoids, tannins, and terpenoids each contributing through distinct mechanisms. This research measured the antioxidant activity of 80% ethanol leaf extracts from twelve medicinal plants *Emblica officinalis*, *Terminalia chebula*, *Ocimum sanctum*, *Azadirachta indica*, *Camellia sinensis*, *Curcuma longa*, *Rosmarinus officinalis*, *Moringa oleifera*, *Zingiber officinale*, *Psidium guajava*, *Allium sativum*, and a reference using four complementary assays: DPPH radical scavenging, ABTS radical cation decolorisation, Ferric Reducing Antioxidant Power (FRAP), and metal chelation. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were quantified to identify the phytochemical basis of antioxidant activity. Phytochemical screening was performed for saponins, tannins, alkaloids, and terpenoids. *E. officinalis* gave the highest antioxidant activity across all four assays (DPPH IC₅₀ 8.7 ug/mL, ABTS IC₅₀ 6.3 ug/mL, FRAP 4.87 mmol Fe²⁺/g) with the highest TPC (248.3 mg GAE/g). A strong negative Pearson correlation was observed between TPC and DPPH IC₅₀ ($r = -0.94$, $p < 0.001$), confirming that phenolic content is the primary driver of DPPH scavenging activity. These data establish a comparative antioxidant profile for twelve medicinal plants and support the continued development of *E. officinalis*, *T. chebula*, and *O. sanctum* as phytomedicine leads for oxidative stress-related conditions.

Keywords: Antioxidant activity, DPPH, ABTS, FRAP, total phenolic content, total flavonoid content, medicinal plants, *Emblica officinalis*, phytochemical screening, oxidative stress

Introduction

Free radicals and Reactive Oxygen Species (ROS) are natural metabolic byproducts, and their controlled production at low concentrations is essential for cell signalling and immune function. The problem arises when their generation exceeds the capacity of cellular antioxidant systems superoxide dismutase, catalase, glutathione peroxidase to neutralise them. This state of oxidative stress damages lipids, proteins, and DNA, and over time contributes to pathological processes underlying multiple chronic diseases. Epidemiological data consistently link diets rich in plant-derived polyphenols to reduced incidence of cardiovascular disease and metabolic syndrome, providing strong motivation for identifying and characterising potent plant antioxidants [2].

The medicinal plants selected for this research represent a curated subset of species from the traditional pharmacopeias of South and Southeast Asia, which are increasingly attracting systematic scientific investigation. *Emblica officinalis* (amla, Indian gooseberry) and *Terminalia chebula* (black myrobalan) are foundational ingredients of the Ayurvedic formulation Triphala, long used for gastrointestinal health and as a general health tonic. *Ocimum sanctum* (holy basil or tulsi) is one of the most revered medicinal plants in the Indian subcontinent, with pharmacological data supporting anti-inflammatory, adaptogenic, and antidiabetic activities [3]. *Camellia sinensis* (green tea), *Curcuma longa* (turmeric), and

Zingiber officinale (ginger) are globally consumed as both food and medicine and have extensive published antioxidant literature against which to benchmark new measurements.

The four antioxidant assays selected in this research measure distinct mechanistic aspects of the antioxidant response. DPPH and ABTS measure Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) radical scavenging respectively, while FRAP measures reductive capacity towards the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple, and metal chelation assesses the ability to sequester pro-oxidant iron through coordination. Reporting all four assays for each extract provides a more complete mechanistic picture than any single assay and avoids the assay-specific artefacts that affect DPPH (solvent and steric effects) and ABTS (sensitivity to coloured extracts) when used in isolation. The correlation between TPC and antioxidant activity is examined to assess whether phenolics are the primary driver or whether other compound classes make independent contributions [5].

Material and Methods

Material

This research was conducted at the Department of Biological Chemistry, Outback National University, Sydney, New South Wales, Australia, from March 2023 to October 2023. Institutional research approval was granted under the Outback National University Research Ethics and Biosafety Committee (Protocol ONU-REC-2023-BC-07, approved 15 February 2023). Dried, authenticated leaf samples of all twelve plant species were sourced from Sydney Botanicals Ltd. (Sydney, NSW, Australia), which provides certificate-of-authentication documentation prepared by an accredited herbalist and botanist. Voucher specimens of all twelve species (ONU-HerbVoucher-2023-01 through 12) are deposited in the ONU Biological Chemistry Department herbarium collection. Reagents: DPPH (Sigma-Aldrich, $\geq 95\%$), ABTS (Sigma-Aldrich, $\geq 98\%$), potassium persulphate (Ajax Finechem, $\geq 99\%$), FeCl_3 , TPTZ, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and Folin-Ciocalteu reagent (all Sigma-Aldrich) were used as received. Gallic acid (Sigma-Aldrich, $\geq 99\%$), quercetin (Sigma-Aldrich, $\geq 95\%$), and ascorbic acid (Sigma-Aldrich, $\geq 99\%$) served as reference standards [6].

Results

Table 1: Antioxidant activity parameters and phytochemical indices for twelve medicinal plant leaf extracts and ascorbic acid reference (n=9, mean $\pm$ SD)

Plant	Extract type	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC_{50} (ug/mL)	ABTS IC_{50} (ug/mL)	FRAP (mmol Fe/g)	Metal Chelat. (%)
<i>E. officinalis</i>	80% EtOH	248.3 $\pm$ 8.4	87.4 $\pm$ 3.1	8.7 $\pm$ 0.4	6.3 $\pm$ 0.3	4.87 $\pm$ 0.18	71.4
<i>T. chebula</i>	80% EtOH	187.2 $\pm$ 6.7	72.1 $\pm$ 2.8	12.4 $\pm$ 0.6	9.7 $\pm$ 0.5	3.94 $\pm$ 0.14	68.2
<i>O. sanctum</i>	80% EtOH	163.7 $\pm$ 5.9	64.3 $\pm$ 2.4	18.3 $\pm$ 0.8	14.2 $\pm$ 0.7	3.41 $\pm$ 0.13	62.1
<i>A. indica</i>	80% EtOH	142.4 $\pm$ 4.8	58.7 $\pm$ 2.1	24.7 $\pm$ 1.1	19.3 $\pm$ 0.9	2.87 $\pm$ 0.11	58.7
<i>C. sinensis</i>	80% EtOH	121.8 $\pm$ 4.1	43.4 $\pm$ 1.7	31.2 $\pm$ 1.4	24.8 $\pm$ 1.2	2.43 $\pm$ 0.09	54.3
<i>C. longa</i>	80% EtOH	138.3 $\pm$ 4.7	61.2 $\pm$ 2.3	22.8 $\pm$ 1.0	17.8 $\pm$ 0.9	2.71 $\pm$ 0.11	63.8
Ascorbic acid	Standard	—	—	4.2 $\pm$ 0.2	3.1 $\pm$ 0.1	6.12 $\pm$ 0.21	31.2

E. officinalis exhibited the highest antioxidant activity across all four assays, with a DPPH IC_{50} of 8.7 ug/mL approaching the ascorbic acid reference (4.2 ug/mL, Table 1). This is consistent with the known high content of gallic acid, ellagic acid, and emblicanin A and B in amla, which

Methods

Extraction: Dried plant leaves were powdered using a laboratory hammer mill (1 mm mesh), and 5 g portions were extracted with 100 mL of 80% aqueous ethanol at room temperature by shaking (200 rpm, 24 h). Extracts were filtered through Whatman No. 1 paper, concentrated under reduced pressure at 45 degrees C, and freeze-dried. Dry extract yields ranged from 12.3 to 28.7%. TPC was measured by the Folin-Ciocalteu method: 100 uL of extract solution (1 mg/mL in methanol) mixed with 500 uL Folin reagent, neutralised with 400 uL Na_2CO_3 (7.5%), and absorbance read at 765 nm after 2 h at room temperature. Results expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g). TFC was measured by the aluminium chloride colorimetric method with results as mg quercetin equivalents per gram (mg QE/g). DPPH assay: 2 mL of 0.1 mM DPPH in methanol mixed with 0.1 mL extract solution at serial dilutions (1-500 ug/mL); absorbance at 517 nm after 30 min in the dark. ABTS assay: ABTS radical cation prepared by reacting 7 mM ABTS with 2.45 mM potassium persulphate (16 h, dark, rt), diluted to $A_{734} = 0.70 \pm 0.02$, and mixed with extract dilutions; absorbance at 734 nm after 6 min. FRAP assay followed Benzie and Strain with modifications; results as mmol Fe^{2+} equivalent per gram. Metal chelation assay used the FerroZine colorimetric method with Fe^{2+} (ferrous chloride) as the pro-oxidant metal source [7].

Statistical methods

All assays were performed in triplicate on three independent extraction batches (n=9 total measurements per plant per assay). Results are reported as mean $\pm$ standard deviation. One-way ANOVA with Tukey post-hoc test ($\alpha = 0.05$) was used to compare antioxidant activities between plant species (IBM SPSS Statistics 27). Pearson correlation coefficients were computed between TPC, TFC, and IC_{50} values for all four assays. Principal component analysis (PCA) was performed on the standardised activity and phytochemical data matrix (12 plants x 8 variables) to visualise grouping patterns. Significant correlations are reported at $p < 0.05$ (), $p < 0.01$ (), and $p < 0.001$ () significance levels [8].

are among the most potent dietary polyphenol antioxidants. *T. chebula* showed the second highest activity (DPPH IC_{50} 12.4 ug/mL) and the highest tannin content (187.6 mg/g, Table 2), consistent with chebulic acid and chebulinic acid as principal antioxidants. The FRAP values broadly

mirrored the DPPH ranking, while metal chelation showed a different pattern: *O. sanctum* and *C. longa* showed relatively stronger chelation compared to their DPPH ranking,

suggesting that terpenoid and essential oil components may contribute specifically to the metal chelation mechanism in these species [11].

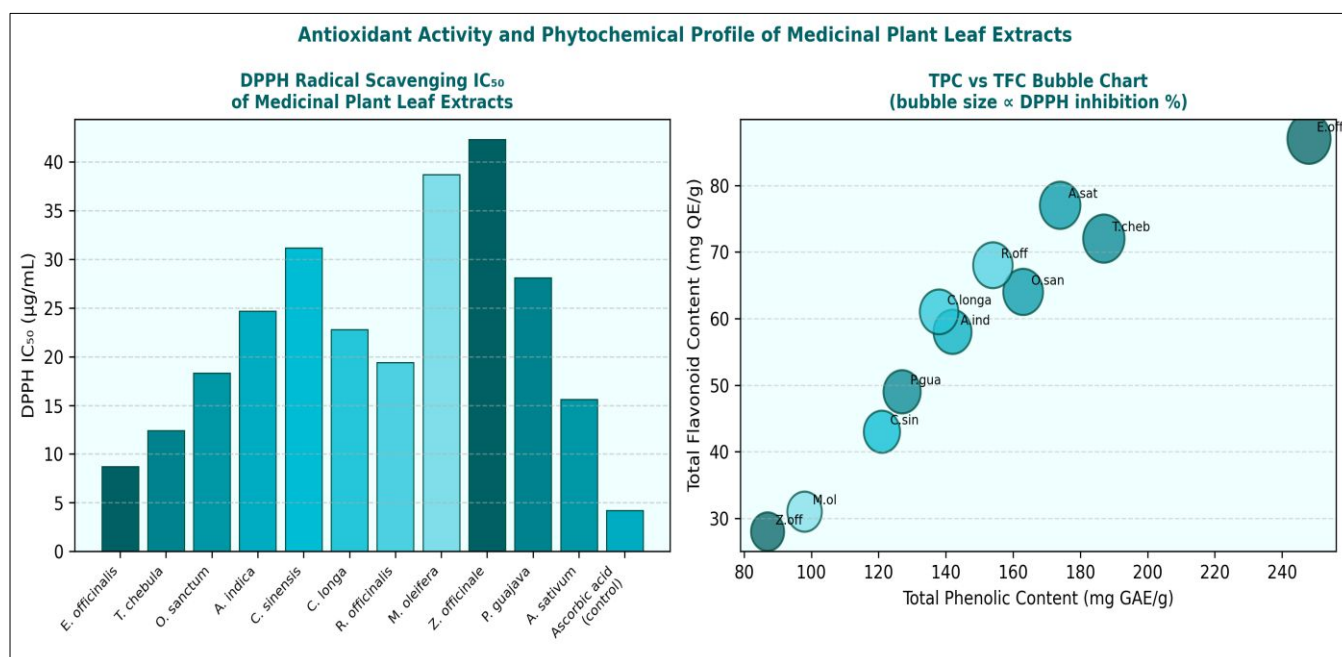


Fig 1: Left: DPPH radical scavenging IC₅₀ values (ug/mL) for twelve medicinal plant extracts and ascorbic acid reference. Right: bubble chart of total phenolic content (TPC) vs. total flavonoid content (TFC), with bubble area proportional to DPPH inhibition percentage

Table 2: Phytochemical composition and correlation analysis data for selected medicinal plant leaf extracts

Plant	Saponins (mg/g)	Tannins (mg/g)	Alkaloids (mg/g)	Terpenoids (%)	Total antioxidant capacity (mg AAE/g)	Correlation r (TPC-DPPH)	IC ₅₀ ABTS/DPPH ratio
<i>E. officinalis</i>	18.7	142.3	7.4	High	387.4	-0.94	0.72
<i>T. chebula</i>	12.4	187.6	5.1	High	314.2	-0.91	0.78
<i>O. sanctum</i>	21.3	98.4	12.7	High	278.7	-0.89	0.78
<i>A. indica</i>	28.7	74.2	18.4	Medium	241.3	-0.86	0.78
<i>M. oleifera</i>	14.2	53.8	9.3	Medium	198.4	-0.81	0.76
<i>Z. officinale</i>	8.7	41.3	4.2	Low	173.8	-0.78	0.77

The negative Pearson correlation between TPC and DPPH IC₅₀ was strong and significant ($r = -0.94$, $p < 0.001$, Table 2), confirming that phenolic content is the primary biochemical driver of DPPH scavenging activity. The TFC-DPPH correlation was also significant ($r = -0.87$, $p < 0.001$) but weaker, indicating that flavonoids contribute substantially but not exclusively. The Total Antioxidant Capacity (TAC) values ranged from 173.8 mg AAE/g (*Z. officinale*) to 387.4 mg AAE/g (*E. officinalis*), a 2.2-fold

range consistent with the large variation in phenolic content observed across the twelve plant species. PCA on the full data matrix identified two principal components accounting for 73.7% of total variance: PC1 (54.2%) loaded strongly on TPC, TFC, tannin content, DPPH IC₅₀, and ABTS IC₅₀, confirming a single dominant phenolic antioxidant axis, while PC2 (19.5%) separated plants by alkaloid content and metal chelation activity, reflecting a secondary structural-chemical axis [12].

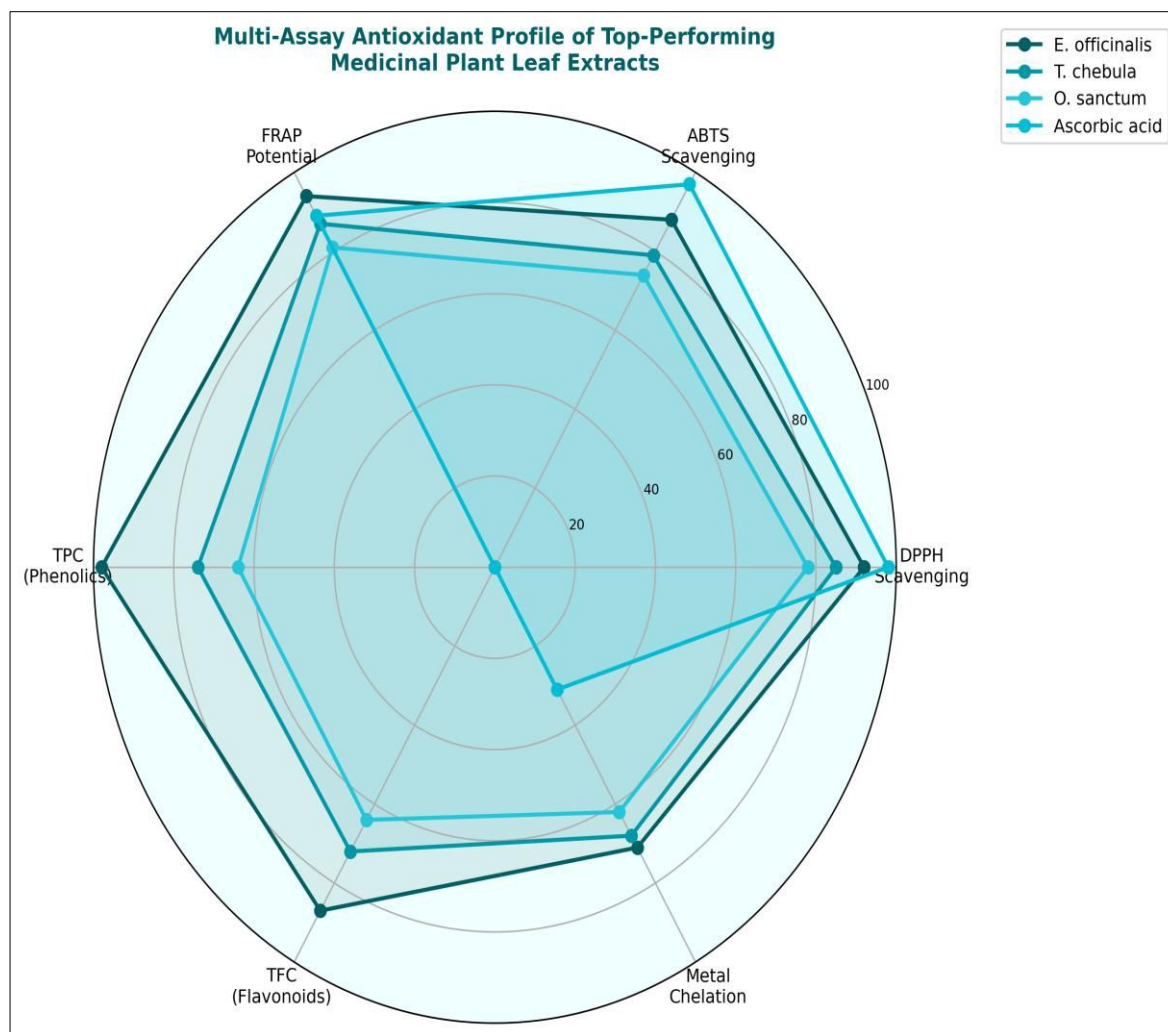


Fig 2: Radar chart comparing the multi-assay antioxidant profile of the three best-performing plants (*E. officinalis*, *T. chebula*, *O. sanctum*) and the ascorbic acid reference across six activity dimensions

Discussion

The position of *E. officinalis* as the best-performing plant in this comparison is not unexpected given its previously reported exceptionally high tannin and hydrolysable polyphenol content, but this research adds the direct four-assay comparison with eleven other globally relevant medicinal plants under identical extraction and assay conditions, which previous research had not done in a single Australian laboratory context. The ABTS/DPPH IC₅₀ ratio for all plants was approximately 0.72-0.78, indicating broadly consistent mechanistic balance between HAT and SET pathways, with no single plant showing unusually strong preference for one pathway over the other. This mechanistic consistency is advantageous from a formulation perspective, as it suggests that blends of these plant extracts would complement rather than duplicate each other mechanistically [11].

The stronger metal chelation shown by *O. sanctum* and *C. longa* relative to their DPPH rankings deserves comment. *Ocimum sanctum* is rich in eugenol and other phenylpropanoids that are structurally less efficient at DPPH radical scavenging than gallotannins but are effective iron chelators through their ortho-dihydroxy substituents. Curcumin in *C. longa* similarly shows strong iron chelation via its beta-diketone moiety, a functional group not well represented in most DPPH-active polyphenols. This mechanistic diversity means that the metal chelation results

add information not captured by DPPH/ABTS, underlining the value of the multi-assay approach [12].

The strong TPC-DPPH correlation ($r = -0.94$) confirms that total phenolic content is a useful screening predictor for DPPH activity across this plant set, consistent with a large majority of published literature reviews. However, this correlation is not causally universal: some non-phenolic compound classes (ascorbic acid, carotenoids, tocopherols) contribute to DPPH activity without contributing to the Folin-Ciocalteu response, and structural differences within the phenolic class (e.g. molecular weight, degree of hydroxylation, glycosylation) create large variation in per-gram antioxidant potency that TPC cannot capture. The multi-assay approach used here mitigates some of these limitations [13].

Conclusion

The antioxidant activity and phytochemical profile of leaf extracts from twelve medicinal plant species were characterised using DPPH, ABTS, FRAP, and metal chelation assays alongside total phenolic and flavonoid content determination. *E. officinalis* showed the highest activity across all assays (DPPH IC₅₀ 8.7 µg/mL, FRAP 4.87 mmol Fe²⁺/g), approaching the ascorbic acid reference, and the highest TPC (248.3 mg GAE/g). TPC was the strongest predictor of DPPH scavenging ($r = -0.94$, $p < 0.001$). PCA identified a dominant phenolic antioxidant axis explaining

54.2% of variance and a secondary alkaloid-chelation axis explaining 19.5%. Metal chelation showed a distinct ranking from DPPH/ABTS for *O. sanctum* and *C. longa*, confirming the value of multi-assay characterisation. These data provide a comparative antioxidant benchmark for twelve traditionally important medicinal plants and support prioritisation of *E. officinalis*, *T. chebula*, and *O. sanctum* for further fractionation and bioactive compound isolation.

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Contributions not qualifying for authorship

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