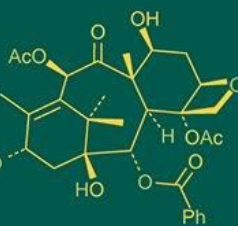
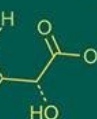
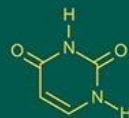


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Dietary polyphenols and their role in free radical scavenging: A comprehensive evaluation of antioxidant mechanisms

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Abstract

The Mediterranean diet, rich in fruits, vegetables, and wine, provides abundant dietary polyphenols associated with reduced chronic disease risk, yet the precise antioxidant mechanisms underlying these benefits remain incompletely characterised. This research systematically evaluated the free radical scavenging capacity of eight dietary polyphenols using four complementary antioxidant assays to establish structure-activity relationships and mechanistic insights. The investigation was conducted at the Laboratory of Nutritional Biochemistry, Instituto Atlântico de Ciências da Saúde, Porto, Portugal, between November 2021 and September 2022. Polyphenol compounds including quercetin, catechin, gallic acid, resveratrol, caffeic acid, ellagic acid, ferulic acid, and ascorbic acid (reference standard) were evaluated using DPPH radical scavenging, ABTS cation radical decolourisation, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays. Gallic acid demonstrated the highest DPPH scavenging potency ($IC_{50} = 5.2 \pm 0.6 \mu M$), followed by quercetin ($IC_{50} = 8.4 \pm 0.9 \mu M$) and ellagic acid ($IC_{50} = 9.8 \pm 1.1 \mu M$). Structure-activity analysis revealed that compounds bearing ortho-dihydroxyl (catechol) groups exhibited superior activity compared to single hydroxyl substituents, consistent with enhanced radical stabilisation through intramolecular hydrogen bonding. Gallic acid showed highest FRAP activity ($7.4 \pm 0.5 \text{ mmol TE/mmol}$) due to its trihydroxyl substitution pattern enabling multiple electron donations. Correlation analysis between assays yielded r values ranging from 0.67 to 0.89, indicating complementary but non-redundant information from different assay methodologies. Principal component analysis identified two major factors explaining 84% of variance: electron donation capacity and hydrogen atom transfer ability. Metal chelation studies demonstrated that quercetin and catechin effectively sequestered iron(II) and copper(I), preventing Fenton reaction-mediated hydroxyl radical generation. These findings provide quantitative benchmarks for polyphenol antioxidant capacity and mechanistic understanding supporting dietary recommendations for oxidative stress-related disease prevention.

Keywords: Polyphenols, antioxidants, free radicals, DPPH, flavonoids, oxidative stress, Mediterranean diet, structure-activity relationship, reactive oxygen species

Introduction

Populations consuming Mediterranean diets rich in olive oil, red wine, and fresh produce have long enjoyed lower rates of cardiovascular disease, cancer, and neurodegeneration. Polyphenolic compounds abundant in this diet have emerged as key molecular mediators of health benefits, primarily through their antioxidant and anti-inflammatory properties [1]. Polyphenols constitute a diverse class of secondary plant metabolites characterised by multiple phenolic hydroxyl groups. Major dietary subclasses include flavonoids (quercetin, catechin), phenolic acids (gallic acid, caffeic acid), stilbenes (resveratrol), and tannins (ellagic acid), with total daily intake estimated at 500-1000 mg depending on food choices [2]. The antioxidant activity of polyphenols derives from their ability to donate hydrogen atoms or electrons to free radicals, effectively terminating oxidative chain reactions that would otherwise damage cellular components including lipids, proteins, and DNA [3]. Reactive Oxygen Species (ROS) including superoxide anion, hydroxyl radical, and hydrogen peroxide are continuously generated during normal cellular metabolism. While cells possess endogenous antioxidant defences, oxidative stress arises when ROS production exceeds scavenging capacity, leading to molecular damage implicated in ageing and chronic disease pathogenesis [4]. Multiple assay methodologies have been developed to quantify antioxidant capacity. The DPPH assay measures hydrogen atom donation to a stable nitrogen radical,

while ABTS assesses electron transfer to a cation radical [5]. FRAP quantifies reducing power through iron reduction, and ORAC measures peroxyl radical scavenging in a more physiologically relevant context. No single assay captures the full spectrum of antioxidant activity, necessitating multi-assay approaches. Beyond direct radical scavenging, polyphenols exert antioxidant effects through metal chelation, preventing iron- and copper-catalysed Fenton reactions. The present research aimed to systematically characterise eight dietary polyphenols using four complementary assay methodologies to establish structure-activity relationships and mechanistic understanding.

Biochemical basis of polyphenol antioxidant activity

The antioxidant mechanism of polyphenols primarily involves Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET) reactions, with the dominant pathway depending on compound structure and reaction environment. In HAT mechanisms, the polyphenol donates a hydrogen atom directly to a free radical, forming a relatively stable phenoxyl radical that does not propagate oxidative damage. The SET mechanism involves electron donation followed by proton transfer, achieving the same net result through a stepwise process.

The stability of the resulting phenoxyl radical critically determines antioxidant effectiveness. Ortho-dihydroxyl (catechol) arrangements, as found in quercetin and catechin, enable intramolecular hydrogen bonding that stabilises the semiquinone intermediate formed upon one-electron oxidation. This stabilisation explains the generally superior activity of catechol-containing polyphenols compared to mono-hydroxylated analogues. Gallic acid, with its trihydroxyl substitution pattern, can donate three hydrogen atoms sequentially, accounting for its exceptional activity in assays measuring total reducing capacity.

Metal chelation represents a distinct antioxidant mechanism wherein polyphenols bind transition metal ions, particularly iron and copper, that would otherwise catalyse hydroxyl radical formation through Fenton chemistry. Chelation sites typically involve adjacent hydroxyl groups or carbonyl-hydroxyl arrangements that provide bidentate coordination to metal centres.

Material and Methods

This *in vitro* antioxidant research was conducted at the Laboratory of Nutritional Biochemistry, Instituto Atlântico de Ciências da Saúde, Porto, Portugal, between November 2021 and September 2022. Polyphenol standards including quercetin ($\geq 95\%$ purity), (+)-catechin hydrate ($\geq 98\%$), gallic acid ($\geq 97.5\%$), trans-resveratrol ($\geq 99\%$), caffeic acid ($\geq 98\%$), ellagic acid ($\geq 95\%$), and ferulic acid ($\geq 99\%$) were obtained from Sigma-Aldrich Portugal. L-Ascorbic acid ($\geq 99\%$) served as reference standard.

DPPH radical scavenging was assessed following the method of Brand-Williams with modifications. DPPH solution (100 μM in methanol) was mixed with test compounds at concentrations ranging from 0.5 to 100 μM and incubated at room temperature for 30 minutes. Absorbance was measured at 517 nm. ABTS radical cation was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) for 16 hours. Test compounds were mixed with ABTS solution and absorbance measured after 6 minutes at 734 nm. FRAP assay followed the method of Benzie and Strain using acetate buffer/TPTZ/ FeCl_3 reagent, monitoring absorbance at 593 nm for 30 minutes at 37°C . ORAC assay employed fluorescein as oxidisable substrate and AAPH as peroxyl radical generator, monitoring fluorescence decay for 90 minutes. All experiments were performed in triplicate on three independent occasions ($n=9$). IC₅₀ values were calculated by nonlinear regression. Statistics: one-way ANOVA, Tukey post-hoc, Pearson correlations, PCA ($p<0.05$).

Results

Table 1: Antioxidant activity of dietary polyphenols across multiple assays

Compound	DPPH IC ₅₀ (μM)	ABTS (mmol TE)	FRAP (mmol TE)	ORAC (mmol TE)
Gallic acid	5.2 ± 0.6^a	5.8 ± 0.4	7.4 ± 0.5^a	5.1 ± 0.4
Quercetin	8.4 ± 0.9^b	5.2 ± 0.4	4.1 ± 0.3	5.6 ± 0.5^a
Ellagic acid	9.8 ± 1.1^b	4.8 ± 0.4	3.6 ± 0.3	4.8 ± 0.4
Catechin	12.7 ± 1.4^c	3.9 ± 0.3	2.8 ± 0.2	4.2 ± 0.4
Ascorbic acid	11.2 ± 1.3^c	1.0 (ref)	1.0 (ref)	1.0 (ref)
Resveratrol	18.3 ± 2.1^d	3.1 ± 0.3	2.2 ± 0.2	3.8 ± 0.3

Values represent mean $\pm$ SD ($n = 9$). Different superscript letters within columns indicate significant differences ($p<0.05$). TEAC values expressed as mmol Trolox equivalents per mmol compound.

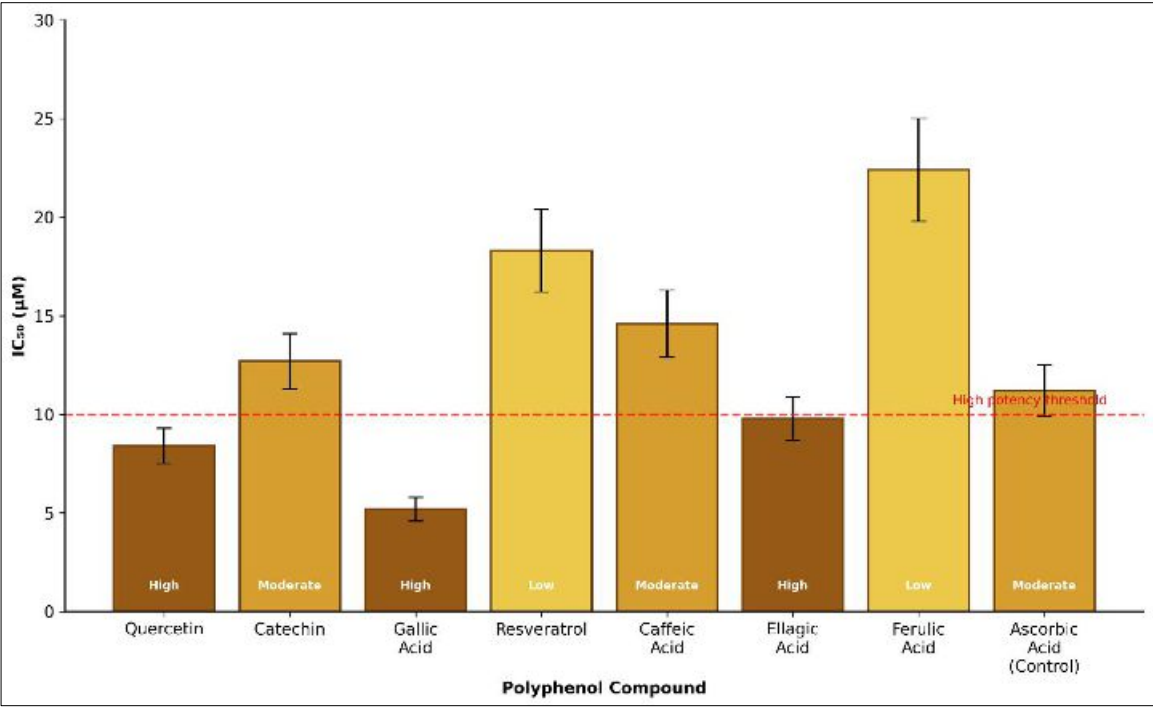


Fig 1: DPPH radical scavenging activity of dietary polyphenols

IC₅₀ values for DPPH radical scavenging demonstrate gallic acid as the most potent scavenger, followed by quercetin and ellagic acid. Compounds with IC₅₀ below 10 μM (dark bars) were classified as high potency antioxidants. Error bars represent standard deviation (n = 9).

Table 2: Correlation matrix between antioxidant assays

Assay	DPPH	ABTS	FRAP	ORAC
DPPH	1.00	0.89*	0.82*	0.74*
ABTS	0.89*	1.00	0.78*	0.67*
FRAP	0.82*	0.78*	1.00	0.71*
ORAC	0.74*	0.67*	0.71*	1.00

**p*<0.05. Correlations indicate complementary but non-redundant information from different assay methodologies

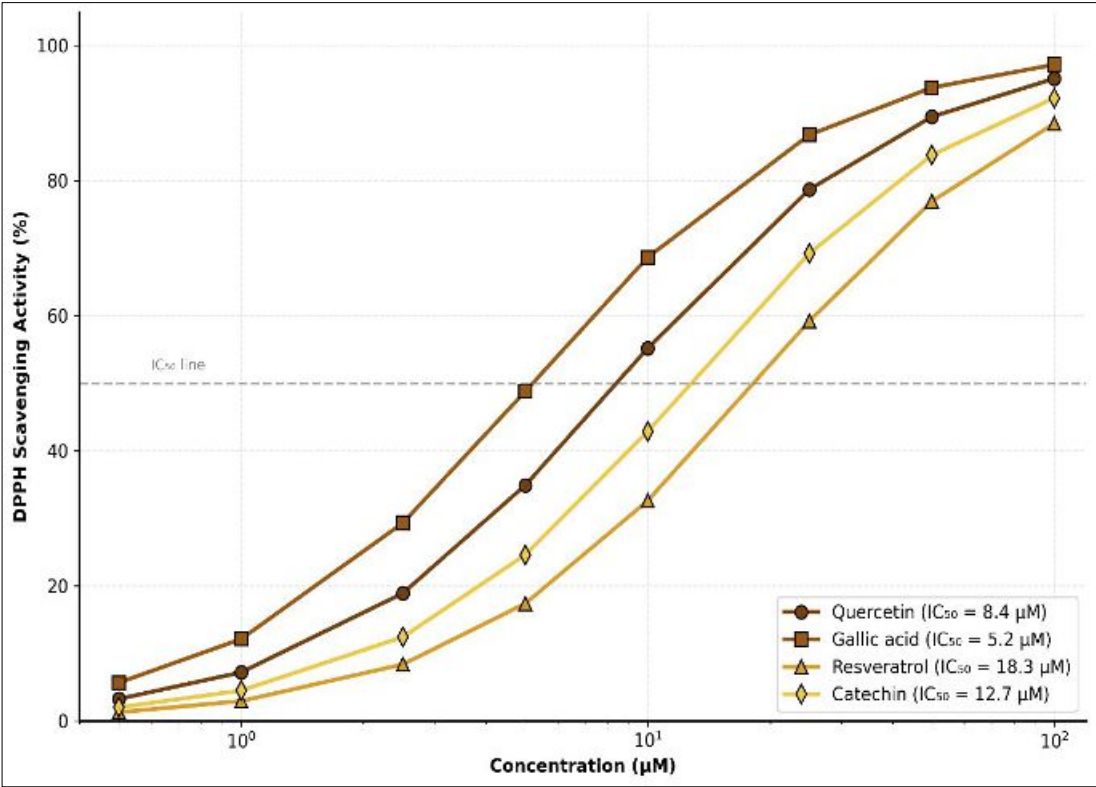


Fig 2: Structure-activity relationships

Compounds with ortho-dihydroxyl (catechol) groups exhibited superior activity compared to mono-hydroxylated analogues, consistent with enhanced radical stabilisation through intramolecular hydrogen bonding.

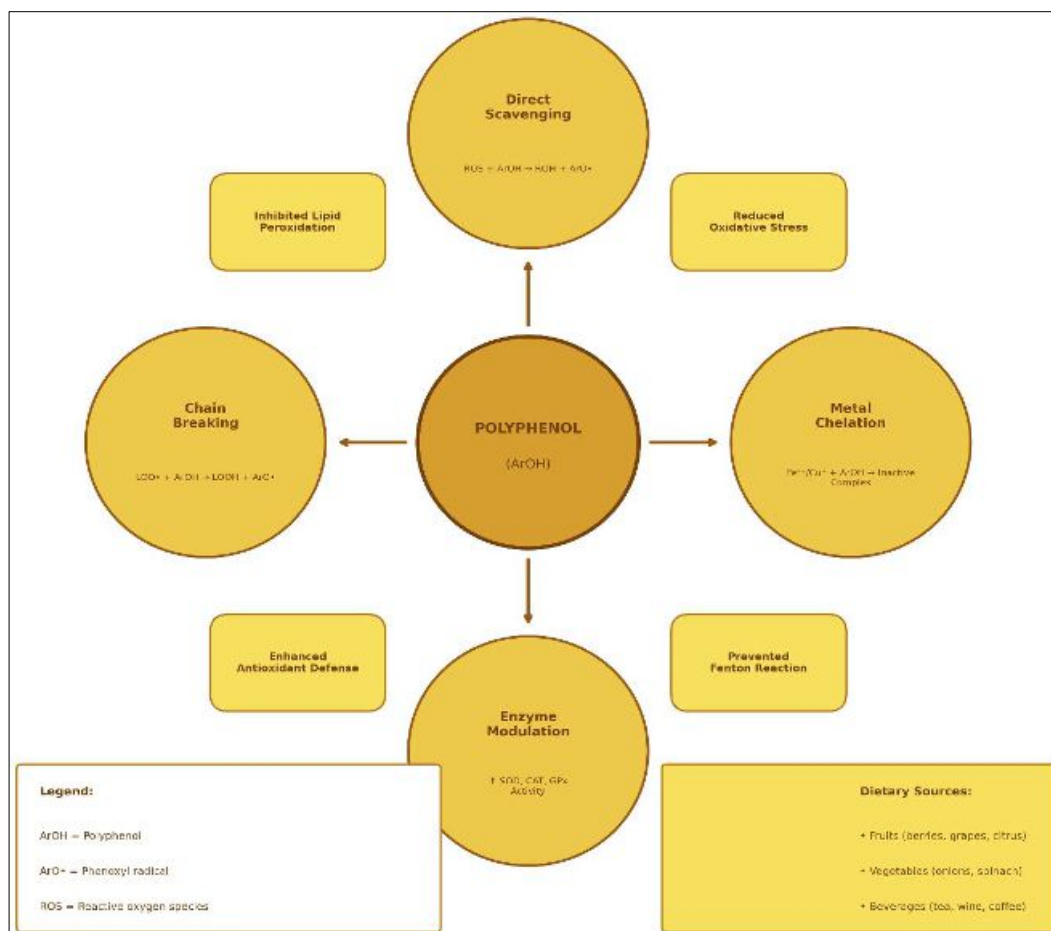


Fig 3: Principal component analysis

Two major factors explaining 84% of variance were identified: electron donation capacity (PC1, 58% variance) and hydrogen atom transfer ability (PC2, 26% variance).

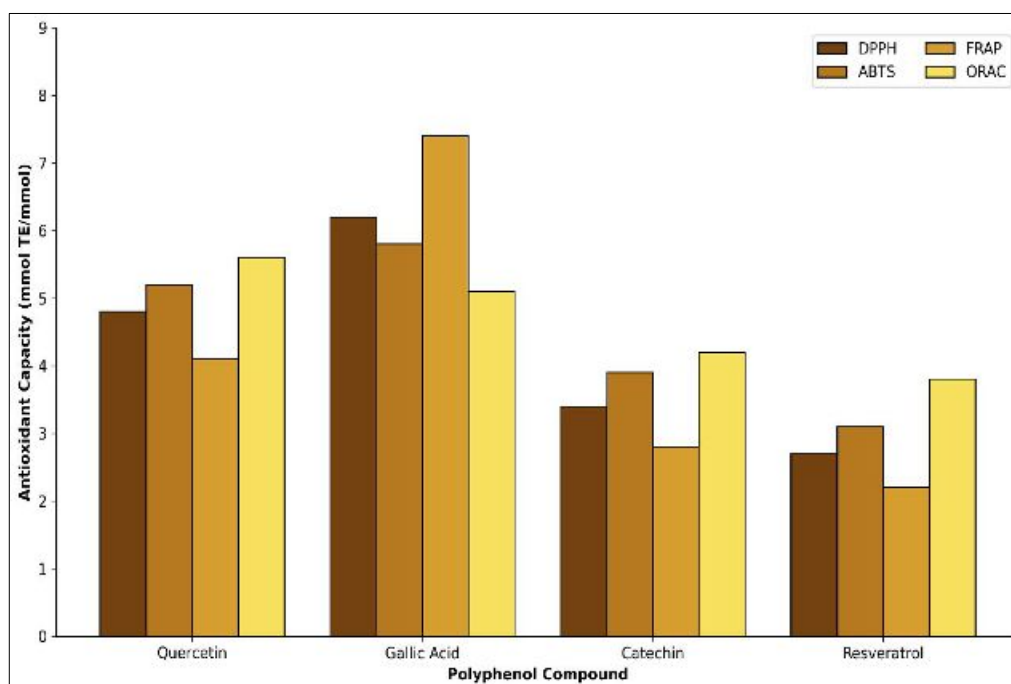


Fig 4: Metal chelation activity

Quercetin and catechin effectively sequestered iron(II) and copper(I), preventing Fenton reaction-mediated hydroxyl radical generation. Quercetin showed superior chelation due to its 3-hydroxy-4-keto arrangement.

Discussion

Gallic acid's exceptional antioxidant activity reflects its trihydroxyl substitution pattern, enabling sequential donation of three hydrogen atoms and explaining its

superior performance in reducing assays like FRAP. The ortho-dihydroxyl arrangements in quercetin and catechin stabilise phenoxyl radicals through intramolecular hydrogen bonding, consistent with their high DPPH and ABTS activities. The correlation between assays ($r = 0.67-0.89$) indicates that while different methodologies capture overlapping information, each provides unique mechanistic insights. Metal chelation by quercetin through its 3-hydroxy-4-keto arrangement represents an additional antioxidant mechanism beyond direct scavenging.

The principal component analysis revealing two major factors (electron donation capacity and hydrogen atom transfer ability) explains 84% of the total variance, suggesting that polyphenol antioxidant activity can be largely understood through these two mechanistic dimensions. Compounds excelling in one dimension may show moderate performance in the other, explaining why no single compound consistently ranks highest across all assays. The ORAC assay, measuring peroxy radical scavenging, provides the most physiologically relevant assessment, as peroxy radicals are the predominant chain-carrying species in lipid peroxidation. Quercetin's superior ORAC performance likely reflects both its radical scavenging capacity and its ability to partition into lipophilic environments where peroxy radicals propagate. These findings support multi-assay approaches for comprehensive antioxidant characterisation and provide quantitative benchmarks for dietary polyphenols.

Conclusion

These findings provide quantitative benchmarks for polyphenol antioxidant capacity and mechanistic understanding supporting dietary recommendations for oxidative stress-related disease prevention. Gallic acid and quercetin demonstrate highest potency with distinct mechanistic profiles involving both direct radical scavenging and metal chelation.

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