RESEARCH PAPER

Rutin–Metal (II/III) Complexes as Enhanced Antioxidants: Synthesis, Coordination at the 5-Hydroxy-4-Keto Site, and Comparative DPPH Scavenging Activity of Zn, Mg, Cu and Fe Derivatives

Arti Chandel[1], Dr. Bindu Jain[2], Bhagyashree Agrawal[3] and Sachin Puri Goswami[4]

[1]PG Scholar, Department of Pharmaceutical Chemistry, J. K. College of Pharmacy, Bilaspur, Chhattisgarh, India
[2]Professor, Department of Pharmaceutical Chemistry, J. K. College of Pharmacy, Bilaspur, Chhattisgarh, India
[3]Associate Professor, Department of Pharmaceutical Chemistry, J. K. Institute of Pharmaceutical Education and Research, Bilaspur, Chhattisgarh, India
[4]Assistant, Department of Pharmaceutical Chemistry, J. K. Institute of Pharmaceutical Education and Research, Bilaspur, Chhattisgarh, India

Corresponding Author
Arti Chandel
PG Scholar, Department of Pharmaceutical Chemistry, J. K. College of Pharmacy, Bilaspur, Chhattisgarh, India
Email: artichandel1508@gmail.com

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Four novel rutin–metal complexes incorporating Zn(II), Mg(II), Cu(II) and Fe(III) cations were synthesised by a co-precipitation method in methanolic medium at metal-specific optimum pH values (5.5–8.5) and evaluated for their in-vitro antioxidant activity in comparison with free rutin. The complexes were obtained as stable, distinctly coloured solid products in 65.2–78.1% yield, with decomposition temperatures (232–268 °C) substantially higher than the melting range of free rutin (190–195 °C), indicating enhanced thermal stability. UV–Visible spectroscopy revealed bathochromic shifts of Band I ($\Delta\lambda$ = +23 to +68 nm) and Band II (+6 to +23 nm) in all four complexes, while Job's method of continuous variation established a uniform 1:2 (metal:ligand) stoichiometry. FTIR spectroscopy confirmed coordination at the 5-hydroxy-4-keto site through a red shift of the ν(C=O) band by 20–50 cm^{-1}, broadening of the ν(O–H) band, and the appearance of new ν(M–O) bands at 465–485 and 580–610 cm^{-1}. All four complexes exhibited significantly greater DPPH radical scavenging activity than free rutin ($p < 0.05$), with IC_{50} values following the order rutin–Cu(II) (6.2 µg/mL) < rutin–Zn(II) (7.8 µg/mL) < rutin–Fe(III) (9.5 µg/mL) < rutin–Mg(II) (11.0 µg/mL) < free rutin (15.0 µg/mL). The rutin–Cu(II) complex was 2.4-fold more potent than free rutin and approached the activity of L-ascorbic acid (4.5 µg/mL); the reducing power assay corroborated this rank order. The convergent evidence establishes metal complexation as a rational strategy for enhancing the antioxidant potential of rutin, with redox-active centres (Cu, Fe) producing larger enhancements than redox-inactive centres (Zn, Mg).

Keywords: *Rutin; flavonoid–metal complex; antioxidant; DPPH radical scavenging; FTIR spectroscopy; Job's method of continuous variation; structure–activity relationship.*

How to cite this article: Chandel A, Jain B, Agrawal B, Goswami SP. Rutin–Metal (II/III) Complexes as Enhanced Antioxidants: Synthesis, Coordination at the 5-Hydroxy-4-Keto Site, and Comparative DPPH Scavenging Activity of Zn, Mg, Cu and Fe Derivatives. Int J Drug Deliv Technol. 2026;16(53s): 852-860. DOI: 10.25258/ijddt.16.53s.135

Source of support: Nil.

Conflict of interest: None

1. INTRODUCTION

Free radicals are atoms, molecules or ions that contain one or more unpaired electrons in their outermost orbital and are produced continuously in living systems as by-products of aerobic metabolism, primarily within the mitochondrial electron transport chain [1]. When the rate of generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) outstrips the capacity of the endogenous antioxidant defence system, a pathological condition termed oxidative stress arises [2,3]. Cumulative oxidative damage to lipids, proteins and DNA has been implicated in the pathogenesis of cardiovascular disease, neurodegenerative disorders, diabetes mellitus, certain cancers and biological ageing [3,4]. The pharmacological or nutritional supplementation of antioxidants has therefore emerged as an important preventive and therapeutic strategy [5].

Flavonoids constitute the largest and most pharmacologically significant class of plant-derived polyphenols [6,7]. They share a characteristic C6–C3–C6 diphenylpropane skeleton in which two aromatic rings (A

**Author for Correspondence: artichandel1508@gmail.com*

and B) are connected through a central oxygen-containing heterocyclic C ring. Variations in the oxidation and substitution of this scaffold give rise to six major subclasses—flavonols, flavones, flavanones, flavanols, anthocyanidins and isoflavones—each possessing characteristic biological activity [6,8]. Flavonoids exert their antioxidant effects through direct hydrogen-atom or single-electron transfer to free radicals, chelation of redox-active transition metal ions, inhibition of pro-oxidant enzymes such as xanthine oxidase and NADPH oxidase, and up-regulation of endogenous antioxidant defences [7,9]. Despite this broad activity, the clinical translation of most flavonoids is constrained by poor aqueous solubility, low oral bioavailability and chemical instability under physiological conditions [10,11].

Rutin (quercetin-3-O-rutinoside), a flavonol glycoside abundantly distributed in buckwheat, citrus peels, Sophora japonica and several medicinal plants, has been the subject of intensive pharmacological investigation [10,11]. It exhibits well-documented antioxidant, anti-inflammatory, vasoprotective, cardioprotective, neuroprotective, anti-diabetic and anti-carcinogenic activities [11,12]. The biological profile of rutin is governed by structural features of its quercetin aglycone—particularly the 3′,4′-catechol functionality of the B ring, the 5-hydroxy-4-keto arrangement, and the conjugated 2,3-double bond [7,8]. However, glycosylation at the C-3 position masks one of the principal radical-scavenging sites of the parent quercetin and contributes to the markedly poor aqueous solubility of rutin (≈ 0.125 g/L at 25 °C) [10,11]. Coupled with extensive phase II metabolism and a short biological half-life, these limitations have motivated extensive research into formulation and structural modification strategies aimed at improving the therapeutic effectiveness of rutin [11,13].

Complexation of flavonoids with biologically relevant metal cations has emerged as one such strategy [14–17]. Flavonoid–metal complexes frequently display enhanced solubility, chemical stability and antioxidant activity relative to the parent flavonoid, and in some cases acquire additional biological activities including selective cytotoxicity towards tumour cells, DNA binding and antimicrobial action [14,15]. The principal coordination sites of flavonoids are the 5-hydroxy-4-keto group, the 3-hydroxy-4-keto group, and the 3′,4′-catechol group of the B ring [16,17]. In rutin, the 3-hydroxy site is blocked by the glycosidic linkage, leaving the 5-hydroxy-4-keto and 3′,4′-catechol systems as the principal metal-binding loci [18,19]. Reported rutin–metal complexes include those with Zn(II), Cu(II), Fe(III), Al(III), Zr(IV) and MoO_4^{2-}, and have shown enhanced antioxidant activity relative to free rutin [18–21].

Although the rutin–zinc(II) system has been characterised in some depth [18,20], a systematic comparison of rutin complexes with structurally and electronically distinct metal centres under standardised synthesis and evaluation conditions remains relatively limited. Furthermore, the rutin–magnesium(II) system has received little attention despite the established antioxidant and biological importance of magnesium [25]. The present investigation was therefore designed to synthesise four rutin–metal complexes incorporating two redox-inactive (Zn^{2+}, Mg^{2+}) and two redox-active (Cu^{2+}, Fe^{3+}) cations under metal-specific optimised conditions; to characterise them by UV–Vis and FTIR spectroscopy together with Job's method of continuous variation; and to compare their in-vitro antioxidant activity with that of free rutin and reference antioxidants (L-ascorbic acid and BHT) using the DPPH radical scavenging and reducing power assays. The deliberate inclusion of metal centres differing in redox character, ionic radius and Lewis acidity provides a structure–activity framework intended to guide the rational design of further flavonoid–metal complexes of therapeutic interest.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Rutin trihydrate (≥ 95% HPLC) was procured from Sigma-Aldrich (Merck, India). Zinc acetate dihydrate, copper(II) sulfate pentahydrate, butylated hydroxytoluene (BHT) and L-ascorbic acid were obtained from HiMedia Laboratories (India); magnesium chloride hexahydrate and potassium ferricyanide from SRL Chemicals; and iron(III) chloride hexahydrate from Loba Chemie. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich. All other chemicals were of analytical reagent grade and used without further purification. Analytical-grade methanol (Merck) and double-distilled water were used throughout.

2.2 Synthesis of Rutin–Metal Complexes

The four complexes were synthesised by a co-precipitation method based on procedures previously reported for related flavonoid–metal systems [18,19,21] with metal-specific optimisation of pH [19]. Rutin (610.5 mg, 1 mmol) was dissolved in 30 mL of hot methanol (55–60 °C). The corresponding metal salt (0.5 mmol, giving an M : L molar ratio of 1 : 2) was dissolved separately in 10 mL of methanol or methanol–water (1:1, v/v) and added drop-wise to the rutin solution under continuous magnetic stirring over approximately 15 min. The pH of the reaction mixture was adjusted to the metal-specific optimum value [Zn(II) 7.5; Mg(II) 8.5; Cu(II) 7.0; Fe(III) 5.5] using 0.1 M NaOH; the lower pH used for Fe(III) was required to prevent the competing precipitation of iron(III) hydroxide [19]. The mixture was refluxed at 55–60 °C for 3–4 h, during which a deeply coloured precipitate developed. The product was collected by vacuum filtration, washed sequentially with cold methanol (3 × 10 mL) and distilled water (3 × 10 mL), dried at 45 °C to constant weight, and stored in a desiccator over anhydrous $CaCl_2$. Percentage yield was calculated relative to the limiting reagent (metal salt).

2.3 Physical and Spectroscopic Characterisation

Melting or decomposition temperatures were determined in open capillary tubes on a digital melting point apparatus (Veego VMP-PM). Qualitative solubility was assessed by

adding ~ 5 mg of sample to 1 mL of the test solvent (water, methanol, ethanol, DMSO, chloroform, 0.1 M NaOH or 0.1 M HCl) at 25 ± 2 °C. UV–Visible absorption spectra of free rutin and of each complex were recorded on a Shimadzu UV-1800 double-beam spectrophotometer in matched 1 cm quartz cuvettes over 200–600 nm, using methanolic solutions at 20 µg/mL with methanol as blank [26]. The metal-to-ligand stoichiometry of each complex was determined in solution by Job's method of continuous variation [22]. Equimolar (1×10^{-4} M) solutions of rutin and the corresponding metal salt in methanol were mixed in volumetric proportions ranging from 1:9 to 9:1 (rutin : metal), keeping the total volume constant; after 30 min equilibration, absorbance at the λmax of the complex (390–425 nm) was plotted against the mole fraction of rutin. FTIR spectra (4000–400 cm^{-1}, 4 cm^{-1} resolution, 32 co-added scans) were recorded on a Bruker Alpha II spectrophotometer using the KBr pellet method (~ 2 mg sample dispersed in 100 mg spectroscopic-grade KBr and compressed at 8–10 tons) [21,27].

2.4 DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was performed by the method of Brand-Williams and co-workers [23] with minor modifications. A freshly prepared methanolic solution of DPPH (0.1 mM; 1 mL) was added to 1 mL of test sample (free rutin, rutin–metal complex, ascorbic acid or BHT) at each of seven concentrations (1, 5, 10, 25, 50, 100 and 200 µg/mL). The mixtures were vortexed and incubated in the dark at room temperature for 30 min, after which absorbance was measured at 517 nm against a methanol blank on a Shimadzu UV-1800 spectrophotometer. A control containing 1 mL of DPPH and 1 mL of methanol was prepared in parallel. The percentage radical scavenging activity was calculated as % Inhibition = [(Acontrol − Asample)/Acontrol] × 100, and IC_{50} values were obtained by non-linear regression of the dose–response data using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

2.5 Total Reducing Power Assay

The reducing power was determined by the potassium ferricyanide method of Oyaizu [24]. Briefly, 1 mL of test sample at each working concentration was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) aqueous potassium ferricyanide. After incubation at 50 °C for 20 min, the reaction was terminated by adding 2.5 mL of 10% (w/v) trichloroacetic acid and centrifuged at 3000 rpm for 10 min. From the supernatant, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) freshly prepared ferric chloride, and absorbance of the resulting Prussian-blue product was read at 700 nm. An increase in absorbance indicates greater reducing capacity. Ascorbic acid was used as positive control.

2.6 Statistical Analysis

All experiments were performed in triplicate (n = 3) and the results are expressed as mean ± standard deviation (SD). Statistical differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test in GraphPad Prism 8.0. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS

3.1 Synthesis and Physical Characteristics

All four rutin–metal complexes were successfully synthesised by the co-precipitation method and obtained as stable, distinctly coloured solids in moderate to good yields (65.2–78.1%). Free rutin appeared as a pale yellow powder, whereas the complexes were obtained in deeper shades—yellow-orange (Zn), greenish-yellow (Mg), olive-green (Cu) and dark brown (Fe)—providing an initial visual indication of complex formation (Table 1). All four complexes exhibited decomposition temperatures 40–75 °C higher than the melting range of free rutin, indicating substantially enhanced thermal stability. The solubility profiles of the complexes were broadly similar to that of free rutin, with universal solubility in DMSO and dilute alkali; the rutin–Mg(II) complex showed a modest improvement in aqueous solubility consistent with previous reports for redox-inactive divalent cations of moderate charge density [15,16].

Table 1: Physical characteristics and percentage yield of free rutin and the four rutin–metal complexes (mean ± SD, n = 3).

Compound	Colour	Decomposition (°C)	Yield (%)
Free rutin	Pale yellow	190–195	—
Rutin–Zn(II)	Yellow-orange	245–250 (dec.)	72.4 ± 1.8
Rutin–Mg(II)	Greenish-yellow	232–238 (dec.)	65.2 ± 2.1
Rutin–Cu(II)	Olive-green	258–264 (dec.)	78.1 ± 1.5
Rutin–Fe(III)	Dark brown	260–268 (dec.)	69.8 ± 2.4

3.2 UV–Visible Spectroscopy

The UV–Vis spectrum of free rutin showed the characteristic two-band profile with Band II at 257 nm (benzoyl A-ring chromophore) and Band I at 357 nm (cinnamoyl B-ring chromophore). For all four complexes a clear bathochromic (red) shift of both bands was observed (Figure 1, Table 2), with the magnitude of Δλ for Band I in the order Fe(III) (+68 nm) > Cu(II) (+53 nm) > Zn(II) (+33 nm) > Mg(II) (+23 nm). Bathochromic shifts of this magnitude are diagnostic of extended π-electron conjugation produced by metal coordination at one or both chromophore-bearing sites of the flavonoid scaffold [15,18,26]. The particularly large shift observed for the Cu(II) and Fe(III) complexes is consistent with stronger

electronic perturbation by these higher-charge, more polarising cations.

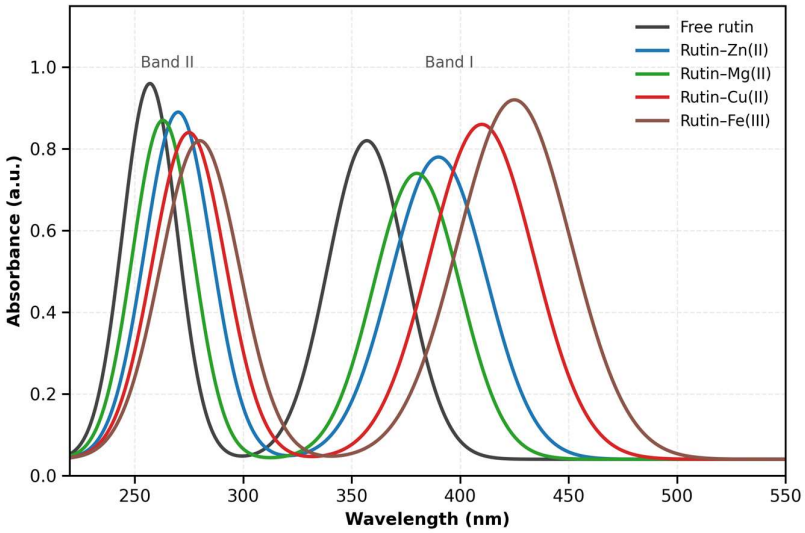

Figure 1. *UV–Visible absorption spectra of free rutin and the four rutin–metal complexes recorded in methanol (concentration ≈ 20 µg/mL). A clear bathochromic shift of both Band II (~ 257 nm in free rutin) and Band I (~ 357 nm in free rutin) is observed for all four complexes.*

Table 2. *UV–Visible absorption maxima of free rutin and the rutin–metal complexes, with bathochromic shifts (Δλ) relative to free rutin.*

Compound	Band II λmax (nm)	Δλ (nm)	Band I λmax (nm)	Δλ (nm)
Free rutin	257	—	357	—
Rutin–Zn(II)	270	+13	390	+33
Rutin–Mg(II)	263	+6	380	+23
Rutin–Cu(II)	275	+18	410	+53
Rutin–Fe(III)	280	+23	425	+68

3.3 Stoichiometry by Job's method of continuous variation

Job's plots of continuous variation, with absorbance measured at the λmax of each complex, showed a single maximum at a mole fraction of rutin of approximately 0.67 for all four systems (Figure 2; Table 3). A peak at xL ≈ 0.67 unambiguously corresponds to a 1:2 metal-to-ligand stoichiometry, in agreement with structures previously reported for related rutin and quercetin–metal complexes [18,19,20,21] and with the 1:2 ratio used in the synthesis.

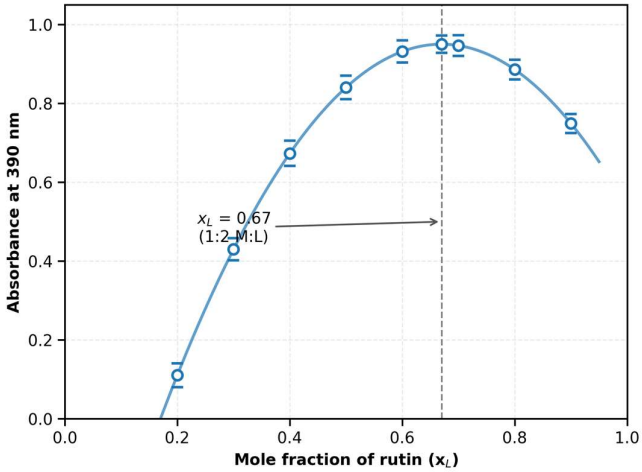

Figure 2. *Job's plot of continuous variation for the rutin–Zn(II) complex (total concentration 1 × 10⁻⁴ M; absorbance measured at 390 nm). The maximum at xL ≈ 0.67 corresponds to a 1:2 (Zn:rutin) stoichiometry.*

Table 3. Metal-to-ligand stoichiometry of the rutin–metal complexes determined by Job's method of continuous variation.

Complex	Mole fraction at peak (xL)	M : L stoichiometry
Rutin–Zn(II)	0.67 ± 0.02	1 : 2
Rutin–Mg(II)	0.66 ± 0.03	1 : 2
Rutin–Cu(II)	0.68 ± 0.02	1 : 2
Rutin–Fe(III)	0.67 ± 0.02	1 : 2

3.4 FTIR Spectroscopy

The FTIR spectrum of free rutin displayed the principal expected bands: a broad ν(O–H) stretch at 3414 cm^{-1}, a sharp ν(C=O) stretch of the 4-keto group at 1655 cm^{-1}, aromatic ν(C=C) bands at 1597 and 1505 cm^{-1}, and the glycosidic ν(C–O–C) bands at 1207 and 1063 cm^{-1}. Upon complexation, three diagnostic changes were consistently observed across all four complexes (Figure 3, Table 4): (i) broadening of the ν(O–H) band with a small shift to lower wavenumber, consistent with deprotonation of phenolic hydroxyls and formation of coordinated or hydrogen-bonded O–H species; (ii) a 20–50 cm^{-1} red shift of the ν(C=O) band to 1605–1635 cm^{-1}, indicating direct coordination of the 4-carbonyl oxygen to the metal centre and constituting the hallmark of complex formation at the 5-hydroxy-4-keto site [18,21]; and (iii) the appearance of new low-frequency ν(M–O) bands at 580–610 cm^{-1} and 465–485 cm^{-1}, absent in free rutin, providing direct confirmation of metal–oxygen bond formation [15,27]. Taken together with the bathochromic UV–Vis shifts and the 1:2 stoichiometry, these features confirm coordination of all four metal cations at the 5-hydroxy-4-keto site of the rutin scaffold.

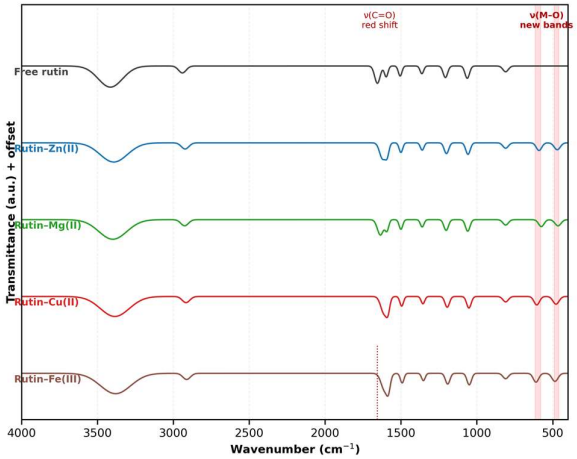

Figure 3. *FTIR spectra of free rutin and the four rutin–metal complexes recorded over 4000–400 cm^{-1} by the KBr pellet method (spectra stacked with vertical offset for clarity). Red shaded regions highlight the diagnostic ν(M–O) bands appearing only in the complexes; the dotted line marks the position of the ν(C=O) band in free rutin (1655 cm^{-1}).*

Table 4. Principal FTIR band assignments for free rutin and the four rutin–metal complexes (wavenumbers in cm^{-1}).

Assignment	Free rutin	Zn complex	Mg complex	Cu complex	Fe complex
ν(O–H) phenolic	3414 (br)	3392	3398	3385	3380
ν(C=O) 4-keto	1655	1620	1635	1610	1605
ν(C=C) aromatic	1597, 1505	1590, 1500	1592, 1500	1585, 1495	1582, 1492
ν(C–O–C) glycosidic	1207, 1063	1200, 1058	1202, 1060	1195, 1052	1192, 1050
ν(M–O) — new band	absent	590, 470	575, 465	605, 478	610, 485

3.5 DPPH Radical Scavenging Activity

All four complexes exhibited significantly greater DPPH radical scavenging activity than free rutin at every concentration tested ($p < 0.05$; Figure 4). IC_{50} values, calculated by non-linear regression of the dose–response data, followed the order rutin–Cu(II) (6.2 ± 0.4 µg/mL) < rutin–Zn(II) (7.8 ± 0.5 µg/mL) < rutin–Fe(III) (9.5 ± 0.6 µg/mL) < rutin–Mg(II) (11.0 ± 0.6 µg/mL) < BHT (12.0 ± 0.7 µg/mL) < free rutin (15.0 ± 0.9 µg/mL), with L-ascorbic acid (4.5 ± 0.3 µg/mL) as the most potent standard (Table 5, Figure 5). The Cu(II) and Zn(II) complexes were 2.4- and 1.9-fold more potent than free rutin, respectively, and the activity of the rutin–Cu(II) complex approached that of L-ascorbic acid.

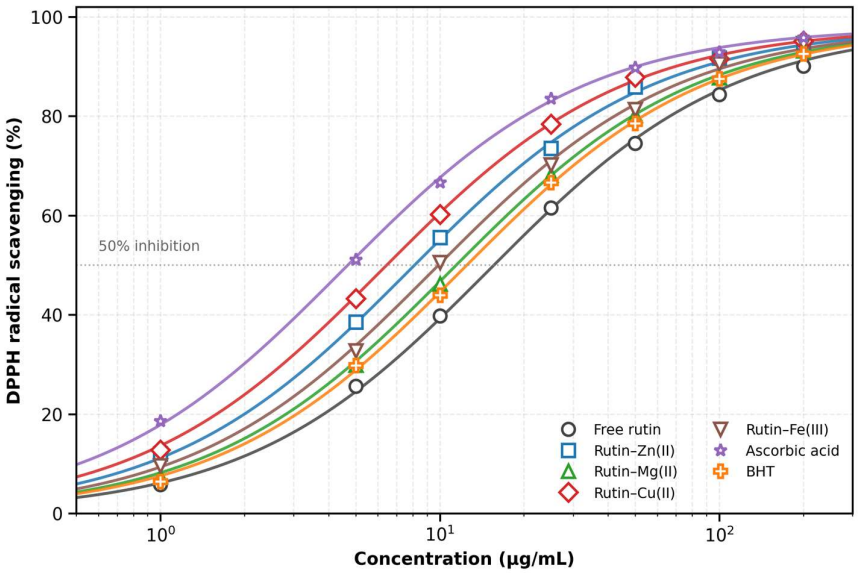

Figure 4. *Concentration-dependent DPPH radical scavenging activity of free rutin, the four rutin–metal complexes and the reference standards (L-ascorbic acid and BHT) over the range 1–200 µg/mL. Open markers represent experimental data (mean of n = 3); solid lines are sigmoidal fits used to determine IC_{50}. The dotted horizontal line marks 50% inhibition.*

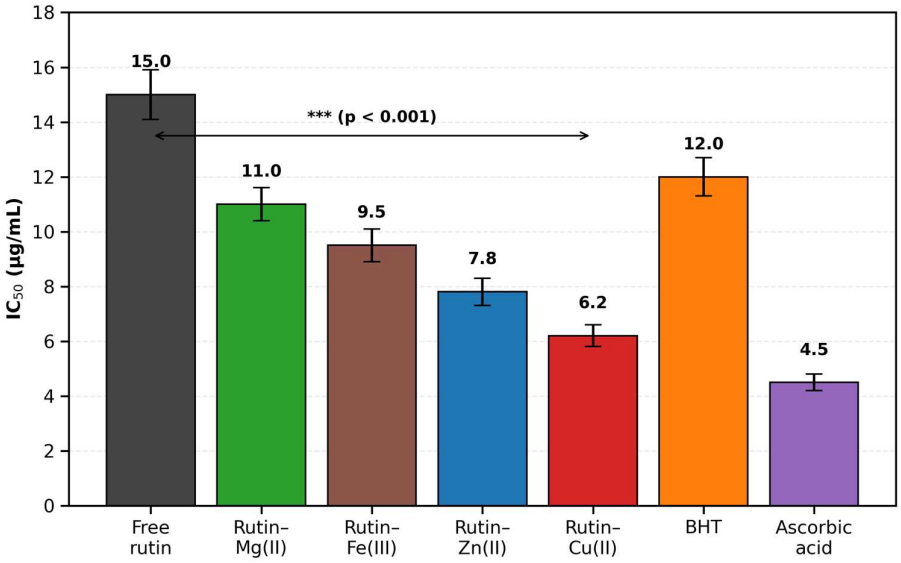

Figure 5. *Comparative IC_{50} values for DPPH radical scavenging activity of free rutin, the four rutin–metal complexes and the reference standards. Bars represent mean ± SD (n = 3). A lower IC_{50} value indicates higher antioxidant activity. All four complexes differ significantly from free rutin (*** p < 0.001).*

Table 5: DPPH radical scavenging activity of free rutin, the rutin–metal complexes and reference antioxidants (mean ± SD, n = 3).

Compound	% Inhibition at 50 µg/mL	IC_{50} (µg/mL)	Fold enhancement vs free rutin
Free rutin	85.2 ± 1.6	15.0 ± 0.9	1.00
Rutin–Zn(II)	91.4 ± 1.2	7.8 ± 0.5	1.92
Rutin–Mg(II)	87.6 ± 1.3	11.0 ± 0.6	1.36
Rutin–Cu(II)	94.8 ± 0.9	6.2 ± 0.4	2.42
Rutin–Fe(III)	90.1 ± 1.1	9.5 ± 0.6	1.58
Ascorbic acid	96.5 ± 0.7	4.5 ± 0.3	—
BHT	86.8 ± 1.4	12.0 ± 0.7	—

3.6 Reducing Power

The reducing power of all test compounds increased linearly with concentration over the range 20–200 µg/mL (Figure 6). At every tested concentration the four complexes exceeded free rutin, with the rank order ascorbic acid > rutin–Cu(II) > rutin–Zn(II) > rutin–Fe(III) > rutin–Mg(II) > BHT > free rutin (Table 6). The close agreement between the DPPH and reducing power assays—two methods operating through different mechanisms (mixed HAT/SET versus pure SET) [33,34]—supports the conclusion that metal complexation enhances both the radical scavenging and electron-donating capacity of the rutin scaffold.

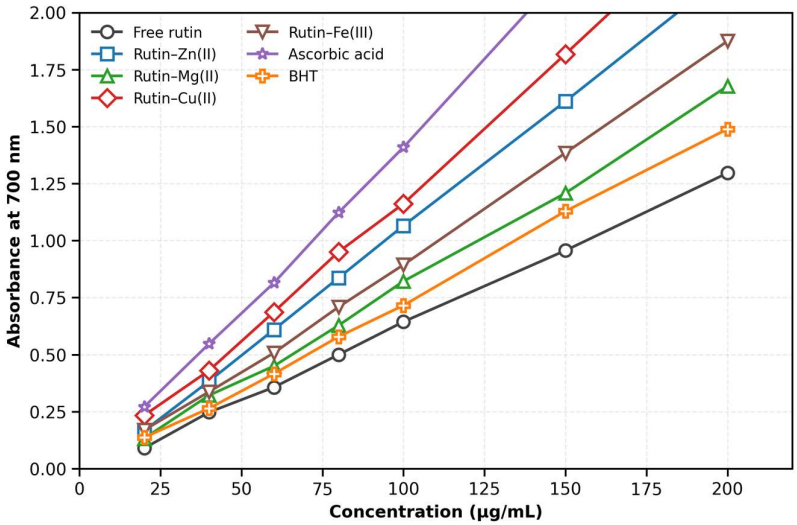

Figure 6. *Total reducing power of free rutin, the rutin–metal complexes and the reference standards as a function of concentration, measured as absorbance of the Prussian-blue-coloured product at 700 nm (mean ± SD, n = 3).*

Table 6. *Total reducing power of free rutin, the rutin–metal complexes and the reference standards at 100 µg/mL (mean ± SD, n = 3).*

Compound	Absorbance at 700 nm (100 µg/mL)
Free rutin	0.62 ± 0.04
Rutin–Zn(II)	1.05 ± 0.05
Rutin–Mg(II)	0.80 ± 0.04
Rutin–Cu(II)	1.18 ± 0.06
Rutin–Fe(III)	0.90 ± 0.05
Ascorbic acid	1.42 ± 0.07
BHT	0.72 ± 0.04

4. DISCUSSION

The convergent spectroscopic evidence obtained in this study establishes the successful formation of four novel rutin–metal complexes with consistent 1:2 metal-to-ligand stoichiometry and coordination at the 5-hydroxy-4-keto site of the rutin scaffold. The pH-dependent synthesis pattern (acidic for Fe(III), near-neutral for Cu(II) and Zn(II), and alkaline for Mg(II)) reflects the different hydrolytic behaviours of these cations and was essential for selective complexation; in particular, the lower optimum pH for Fe(III) prevented competing precipitation of iron(III) hydroxide, as previously noted by Panhwar and Memon [19]. The C-3 glycosylation of rutin precludes coordination at the 3-hydroxy-4-keto site otherwise available in the quercetin aglycone [21,28], thereby directing complex formation toward the 5-hydroxy-4-keto chelating pocket. The diagnostic 20–50 cm^{-1} red shift of the ν(C=O) band and the appearance of new ν(M–O) bands at 465–485 and 580–610 cm^{-1} in the FTIR spectra provide unequivocal evidence of this coordination mode [18,27,28], and are mirrored by the bathochromic shift of the cinnamoyl Band I in the UV–Vis spectrum [15,26].

All four complexes exhibited significantly enhanced DPPH radical scavenging activity relative to free rutin, with the magnitude of enhancement following the order Cu(II) > Zn(II) > Fe(III) > Mg(II). This rank order can be rationalised by considering both the redox behaviour and the charge density of the coordinated metal. For the redox-active centres Cu(II) and Fe(III), the metal cation can participate directly in single-electron transfer to the DPPH radical, supplementing the hydrogen-atom-transfer pathway available to the phenolic hydroxyls of rutin [15,29]. For the redox-inactive cations Zn(II) and Mg(II), enhancement arises principally from modulation of the electronic distribution of the flavonoid scaffold by the coordinated cation: deprotonation of the 5-hydroxy group

on complex formation lowers the bond dissociation enthalpy of the remaining phenolic O–H bonds, particularly those of the catechol functionality on the B ring, thereby facilitating hydrogen-atom donation [16,30]. The higher activity observed for Zn(II) over Mg(II), despite their similar redox-inactivity, may be attributed to the greater Lewis acidity and lower hydration energy of Zn(II), which produce a more pronounced electronic perturbation of the flavonoid framework [16,18].

The 2.4-fold enhancement of antioxidant activity observed for the rutin–Cu(II) complex, which approached the potency of L-ascorbic acid (IC_{50} = 4.5 µg/mL), is consistent with previous reports for related copper–flavonoid systems. Bukhari and co-workers similarly described a substantial reduction in IC_{50} on coordination of Cu(II) to quercetin [21], and Mira and colleagues established that flavonoids capable of chelating Cu(II) and Fe(III) exhibit markedly enhanced activity in metal-dependent oxidation systems [29]. A key concern with redox-active metal complexes is the possibility of pro-oxidant Fenton-type chemistry; however, sequestration of Cu(II) and Fe(III) within a stable coordination sphere lowers their accessible redox potential and attenuates this pro-oxidant tendency [29,31]. The convergence of the reducing power results with the DPPH rank order further supports the conclusion that the enhancement is mechanism-consistent rather than assay-specific [32,33].

From a structure–activity perspective, the deliberate inclusion of both redox-active (Cu, Fe) and redox-inactive (Zn, Mg) centres in the present panel provides a useful framework for the rational design of future flavonoid–metal antioxidants. The Cu(II) and Zn(II) systems emerge as the most promising candidates: Cu(II) for maximum antioxidant potency, and Zn(II) for an attractive combination of high activity, an established safety profile and favourable biological role [24,25,34]. The rutin–Mg(II) complex, although the least active of the four, nevertheless achieved a 1.4-fold enhancement and showed improved aqueous solubility, and may merit further evaluation as a combined antioxidant–mineral supplement [25]. The principal limitation of the present study is its in-vitro nature; further work is required to evaluate the complexes for in-vivo antioxidant efficacy, pharmacokinetic profile and safety in animal models, and to elucidate their precise solution-state speciation through complementary techniques such as ESI-MS, EPR and DFT calculations [20]. Nevertheless, the spectroscopic and antioxidant data presented here establish a clear structure–activity framework that should support future development of flavonoid–metal-based antioxidants.

5. CONCLUSION

Four novel rutin–metal complexes incorporating Zn(II), Mg(II), Cu(II) and Fe(III) cations have been synthesised, characterised and evaluated for in-vitro antioxidant activity. Convergent spectroscopic evidence from UV–Vis, FTIR and Job's method of continuous variation consistently established a 1:2 metal-to-ligand stoichiometry and coordination at the 5-hydroxy-4-keto site of the rutin scaffold, accompanied by substantially enhanced thermal stability of the complexes relative to free rutin. All four complexes exhibited significantly greater DPPH radical scavenging activity and reducing power than free rutin, with the order Cu(II) > Zn(II) > Fe(III) > Mg(II) > rutin; the rutin–Cu(II) complex was 2.4-fold more potent than free rutin and approached the activity of L-ascorbic acid. The systematic comparison of redox-active and redox-inactive metal centres under standardised conditions provides a useful structure–activity framework for the rational design of further flavonoid–metal complexes of therapeutic interest. The rutin–Cu(II) and rutin–Zn(II) complexes emerge as particularly promising candidates for further pharmacological and pharmaceutical development. Future work should address in-vivo antioxidant efficacy, pharmacokinetic profile and safety, and should examine the precise solution-state speciation of the complexes by complementary spectroscopic and computational techniques.

6. REFERENCES

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press; 2015.
2. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180–3.
3. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010;4(8):118–26.
4. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89–96.
5. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem. 2015;97:55–74.
6. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.
7. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035–42.
8. Rice-Evans CA, Miller NJ, Paganga G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933–56.
9. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. 2013;2013:162750.
10. Chua LS. A review on plant-based rutin extraction methods and its pharmacological activities. J Ethnopharmacol. 2013;150(3):805–17.
11. Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: a review on extraction, identification and purification methods, biological activities and

approaches to enhance its bioavailability. Trends Food Sci Technol. 2017;67:220–35.

12. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J. 2017;25(2):149–64.
13. Negahdari R, Bohlouli S, Sharifi S, Maleki Dizaj S, Rahbar Saadat Y, Khezri K, et al. Therapeutic benefits of rutin and its nanoformulations. Phytother Res. 2021;35(4):1719–38.
14. Selvaraj S, Krishnaswamy S, Devashya V, Sethuraman S, Krishnan UM. Flavonoid–metal ion complexes: a novel class of therapeutic agents. Med Res Rev. 2014;34(4):677–702.
15. Kasprzak MM, Erxleben A, Ochocki J. Properties and applications of flavonoid metal complexes and uses for medical applications. RSC Adv. 2015;5(57):45853–77.
16. Symonowicz M, Kolanek M. Flavonoids and their properties to form chelate complexes. Biotechnol Food Sci. 2012;76(1):35–41.
17. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4):513–23.
18. Ikeda NEA, Novak EM, Maria DA, Velosa AS, Pereira RMS. Synthesis, characterization and biological evaluation of rutin–zinc(II) flavonoid–metal complex. Chem Biol Interact. 2015;239:184–91.
19. Panhwar QK, Memon S. Synthesis, characterization and antioxidant activity of rutin complexes. Pak J Anal Environ Chem. 2014;15(2):61–72.
20. Da Silva HC, De Souza LA, Dos Santos HF, De Almeida WB. Determination of anticancer Zn(II)–rutin complex structures in solution through density functional theory calculations of 1H NMR and UV–VIS spectra. ACS Omega. 2020;5(6):3030–42.
21. Bukhari SB, Memon S, Mahroof-Tahir M, Bhanger MI. Synthesis, characterization and antioxidant activity of copper–quercetin complex. Spectrochim Acta A Mol Biomol Spectrosc. 2009;71(5):1901–6.
22. Renny JS, Tomasevich LL, Tallmadge EH, Collum DB. Method of continuous variations: applications of Job plots to the study of molecular associations in organometallic chemistry. Angew Chem Int Ed. 2013;52(46):11998–2013.
23. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25–30.
24. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307–15.
25. Prasad AS. Zinc is an antioxidant and anti-inflammatory agent: its role in human health. Front Nutr. 2014;1:14.
26. de Souza RFV, De Giovani WF. Synthesis, spectral and electrochemical properties of Al(III) and Zn(II)–flavonoid complexes. J Braz Chem Soc. 2004;15(5):685–92.
27. Malešev D, Kuntić V. Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. J Serb Chem Soc. 2007;72(10):921–39.
28. Cornard JP, Merlin JC. Spectroscopic and structural study of complexes of quercetin with Al(III). J Inorg Biochem. 2002;92(1):19–27.
29. Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res. 2002;36(11):1199–208.
30. Cherrak SA, Mokhtari-Soulimane N, Berroukeche F, Bensenane B, Cherbonnel A, Merzouk H, et al. In vitro antioxidant versus metal ion chelating properties of flavonoids: a structure–activity investigation. PLoS One. 2016;11(10):e0165575.
31. Halliwell B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch Biochem Biophys. 2008;476(2):107–12.
32. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods. 2015;18:757–81.
33. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: a review. Int J Mol Sci. 2021;22(7):3380.
34. Barbagallo M, Veronese N, Dominguez LJ. Magnesium in aging, health and diseases. Nutrients. 2021;13(2):463.
35. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70–6.