RASĀYAN *J. Chem.*
Vol. 19 | No. 1 | 348-354 | January-March | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ROLE OF PHENOLIC HYDROXYL GROUPS IN BOOSTING ANTIOXIDANT ACTIVITY OF UNSATURATED β-KETOESTERS AND THEIR Cu(II) COMPLEXES

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ABSTRACT

The antioxidant activity of nine unsaturated β-ketoesters and their Cu(II) complexes was evaluated by the DPPH radical scavenging method. Compounds bearing phenolic –OH groups exhibited higher radical scavenging activity, which increased with both the number of –OH groups and the degree of electron delocalisation. Among the ligands, HL^2, HL^3, HL^6, and HL^8 exhibited notable activity in the order $HL^2 > HL^3 > HL^6 > HL^8$. Coordination with Cu(II) further enhanced antioxidant efficiency, attributed to the $[CuL_2]$ stoichiometry that doubles the active phenolic sites and enables Cu(II)/Cu(I) redox cycling. Steric effects also influenced radical-scavenging capacity, emphasising the critical roles of phenolic substitution and metal complexation in modulating antioxidant efficacy. The study provides insights into structure–activity relationships in synthetic curcuminoid analogues and highlights β-ketoester-based metal complexes as promising antioxidant agents, supporting Sustainable Development Goal 3 (Good Health and Well-being) by contributing to the development of potential therapeutic antioxidants.

Keywords: Unsaturated β-ketoesters, Cu(II) Complexes, Phenolic Hydroxyl Groups, Antioxidant Activity, DPPH Method, Structure–Activity Relationship, Curcuminoid Analogues.

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INTRODUCTION

Unchecked reactive oxygen species (ROS) can tip the delicate balance of cellular redox homeostasis, leading to oxidative stress, a key factor in chronic and degenerative diseases such as cancer, cardiovascular disorders, and diabetes.[1] This growing awareness of oxidative damage has fuelled extensive research into antioxidant compounds capable of neutralising free radicals. Among these, curcuminoids—a class of naturally occurring β-diketones—have attracted significant attention due to their broad biological activities, including anti-inflammatory, antioxidant, and anticancer properties.[2–5] However, their limited stability and bioavailability have prompted the development of synthetic curcuminoid analogues with enhanced structural and functional properties.[5,6] Unsaturated β-ketoesters represent a promising class of curcuminoid analogues.

These molecules contain a β-dicarbonyl moiety that facilitates electron delocalisation and intramolecular hydrogen transfer, which are critical for radical-scavenging activity.[7,8] Introduction of phenolic hydroxyl groups further enhances antioxidant potential by enabling efficient hydrogen atom donation and resonance stabilisation.[9] Despite extensive studies on β-diketones, systematic investigations on unsaturated β-ketoesters, particularly regarding the effects of substituents and metal coordination on antioxidant activity, remain scarce.[5,6,10] The present study addresses this gap by evaluating the antioxidant behaviour of nine unsaturated β-ketoesters and their Cu(II) complexes using the DPPH radical scavenging assay.

The work focuses on the roles of phenolic substitution, electronic delocalisation, and Cu(II) coordination in modulating antioxidant activity. Enhanced activity observed in Cu(II) complexes is attributed to the $[CuL_2]$ stoichiometry, which doubles active phenolic sites and enables Cu(II)/Cu(I) redox cycling. This research also aligns with Sustainable Development Goal 3 (Good Health and Well-being) by contributing to the identification of potential therapeutic antioxidants, and SDG 12 (Responsible Consumption and Production) through the rational design of efficient and sustainable synthetic compounds.

http://doi.org/10.31788/RJC.2026.1918560

EXPERIMENTAL

Material and Methods

All chemicals and solvents used were of analytical reagent grade and employed without further purification. Methyl acetoacetate, boric anhydride, copper(II) acetate monohydrate, and aromatic aldehydes were obtained from Merck, India. The aromatic aldehydes included benzaldehyde, 3,4-dihydroxybenzaldehyde, vanillin, 4-nitrobenzaldehyde, 4-methoxybenzaldehyde, 4-hydroxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 2-hydroxynaphthaldehyde, and indole-3-carbaldehyde. Tri-sec-butyl borate was purchased from Fluka, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) used for antioxidant activity studies was obtained from Sigma-Aldrich. Analytical-grade ethanol and methanol were used as solvents for synthesis, recrystallisation, and spectroscopic studies. Melting points were determined in open capillaries and are uncorrected. The antioxidant activity of the unsaturated β-ketoesters and their Cu(II) complexes was evaluated by the DPPH radical scavenging method. A 0.1 mM methanolic solution of DPPH was prepared, and the absorbance was measured at 516 nm using a JASCO V-550 UV–Visible spectrophotometer after 30 min of incubation in the dark. Ascorbic acid served as the reference standard, and all determinations were carried out in triplicate.

Synthesis of Unsaturated β-ketoesters, HL

The unsaturated β-ketoesters (HL^1–HL^9) were prepared using a modified Pabon method.[2] In this modification, methyl acetoacetate was employed as the β-dicarbonyl component instead of acetylacetone.[7,8] In a typical procedure, equimolar quantities of methyl acetoacetate and the corresponding aromatic aldehyde were mixed with boric anhydride and tri-sec-butyl borate, followed by the addition of a catalytic amount of n-butylamine. The reaction mixture was stirred and refluxed under controlled temperature until product formation was complete, as confirmed by thin-layer chromatography (TLC). After completion, the mixture was cooled to room temperature, and the solid obtained was filtered, washed successively with water and cold ethanol, and recrystallised from methanol to afford chromatographically pure ligands. The structures of the synthesised unsaturated β-ketoesters are shown in Fig.-1.

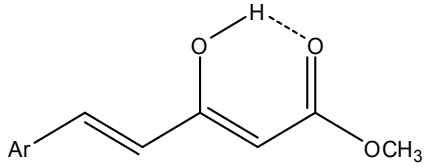

Fig.-1: Structure of the Unsaturated β-ketoesters (HL)

Ar = Phenyl (HL^1); 3,4-dihydroxyphenyl (HL^2); 4-hydroxy-3-methoxyphenyl (HL^3); 4-nitrophenyl (HL^4); 4-methoxyphenyl (HL^5); 4-hydroxyphenyl (HL^6); 3,4-dimethoxyphenyl (HL^7); 2-hydroxynaphthyl (HL^8); 3-Indolyl (HL^9)

Synthesis of Complexes, [CuL_2]

The Cu(II) complexes of the ligands were prepared following literature procedures.[7,8] Two equivalents of ligand react with one equivalent of Cu(II) to form [CuL_2] complexes. In a typical synthesis, two equivalents of the ligand were dissolved in ethanol and added dropwise to an ethanolic solution of Cu(II) acetate monohydrate with continuous stirring at room temperature. The reaction mixture was stirred for 2–3 hours until the formation of the complex was complete, as evidenced by a change in colour. The resulting Cu(II) complex was separated as a solid, which was filtered, washed successively with cold ethanol and diethyl ether, and dried under vacuum. The Cu(II) complexes with [CuL_2] stoichiometry of these ligands are depicted in Fig.-2.

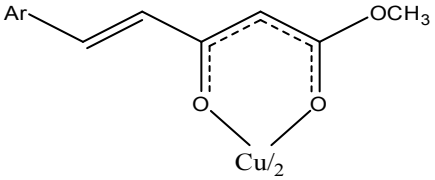

Fig.-2: Structure of the Cu(II) Complexes of Unsaturated β-ketoesters, [CuL_2]

Antioxidant Studies

The antioxidant activities of the synthesised ligands and their Cu(II) complexes were evaluated using the DPPH free radical scavenging method.[11,12] A 0.1 mM methanol stock solution of DPPH was prepared, and 2 mL of this solution was mixed with varying volumes of the sample solution (1–200 μg/mL), and the mixture was made up to 10 mL with methanol. The mixtures were kept in the dark for 30 minutes, and the absorbance was recorded at 516 nm. The percentage inhibition of DPPH was calculated, and the EC_{50} values were determined from the calibration curve. For compounds with lower antioxidant activity, measurements were adjusted accordingly. Further parameters, such as Anti-Radical Power (ARP), Stoichiometric Value (SV), and the number of DPPH molecules reduced per mole of compound (NRD), were also calculated.

RESULTS AND DISCUSSION

The antioxidant activities of nine unsaturated β-ketoesters (HL^1–HL^9, Fig.-1) and their Cu(II) complexes ([CuL_2], Fig.-2) were evaluated using the DPPH free radical scavenging method and summarised in Tables 1–3, and the concentration–response curves are presented in Fig.-3–10. Among the tested ligands, HL^2, HL^3, HL^6 and HL^8 exhibited significant antioxidant activity, while HL^1, HL^4, HL^5, HL^7, and HL^9 showed comparatively low or negligible activity. The activity order of the active ligands is $HL^2 > HL^3 > HL^6 > HL^8$. This trend indicates that the presence and position of phenolic hydroxyl groups play a decisive role in DPPH radical scavenging.[13] The higher activity of HL^2 is attributed to the presence of two phenolic –OH groups, which can participate in efficient hydrogen atom transfer (HAT). In HL^3, the adjacent $–OCH_3$ group stabilises the phenoxy radical through resonance, resulting in moderately high activity. HL^6, having only one –OH group without a stabilising $–OCH_3$ substituent, exhibits slightly lower activity, whereas HL^8 shows reduced activity, possibly due to steric hindrance from the bulky naphthyl group, which limits radical interaction.[14] The Cu(II) complexes of these ligands follow the same order of activity as their parent compounds but exhibit higher overall antioxidant efficiency. This enhancement may arise from the presence of two equivalents of phenolic groups per [CuL_2] complex and the possible participation of the Cu(II)/Cu(I) redox couple, which facilitates electron or hydrogen transfer during radical quenching. The data clearly demonstrate the synergistic effect of metal coordination on radical stabilisation and scavenging capacity. The remaining ligands (HL^1, HL^4, HL^5, HL^7, and HL^9) and their Cu(II) complexes show very low antioxidant activities, consistent with the absence of phenolic hydroxyl groups. Although the energy required to release hydrogen from enolic and phenolic –OH groups is similar, the phenolic group contributes more effectively to antioxidant behaviour. Among these, HL^9 shows slightly better activity than expected, possibly due to the presence of the –NH group of the indolyl moiety, which can also participate in hydrogen donation.[14] HL^4 is practically inactive, and its Cu(II) complex shows no measurable activity.

Quantitative evaluation of the antioxidant behaviour was carried out by determining EC_{50}, Anti-Radical Power (ARP), Stoichiometric Value (SV), and the number of DPPH molecules reduced per mole of compound (NRD) (Table-3). These parameters further confirm the observed structure–activity relationship, where the number and electronic nature of phenolic substituents govern radical scavenging efficiency. The graphical representations (Fig.-3 to 10) clearly illustrate the concentration-dependent inhibition of DPPH radicals by both ligands and their Cu(II) complexes. Overall, the findings highlight that phenolic functionality and Cu(II) coordination significantly enhance antioxidant potential. The study contributes to the understanding of structure–activity relationships in curcuminoid analogues and supports the design of new metal-based antioxidant agents relevant to oxidative stress mitigation and related biomedical applications.

Table-1: Antioxidant Assay of the Active Unsaturated β-ketoesters

Compound	Concentration (μg/mL)	Absorbance	% Difference	% Inhibition	EC_{50} (μg/mL)
HL^2	0	1.0628	-	-	38.95
	14.64	0.8324	78.3	21.7	
	29.28	0.6310	59.4	40.6	
	43.92	0.4690	44.2	55.8	
	58.56	0.2500	23.5	76.5	

	73.20	0.0958	9.1	90.9	
HL^3	0	0.695	-	-	75.17
	31.2	0.673	96.8	3.2	
	46.8	0.549	79.0	21.0	
	62.4	0.423	60.9	39.1	
	78.0	0.325	46.8	53.2	
	93.6	0.235	33.8	66.2	
	109.2	0.107	15.5	84.5	
HL^6	0	0.689	-	-	114.89
	39.9	0.568	82.4	17.7	
	79.8	0.445	64.6	35.4	
	119.7	0.328	47.6	52.4	
	159.6	0.204	29.6	70.4	
	199.5	0.124	15.1	81.9	
HL^8	0	0.697	-	-	190.37
	51.2	0.603	86.5	13.5	
	102.4	0.503	72.2	27.8	
	153.6	0.409	58.8	41.2	
	204.8	0.331	47.6	52.4	
	256.0	0.211	30.3	69.7	

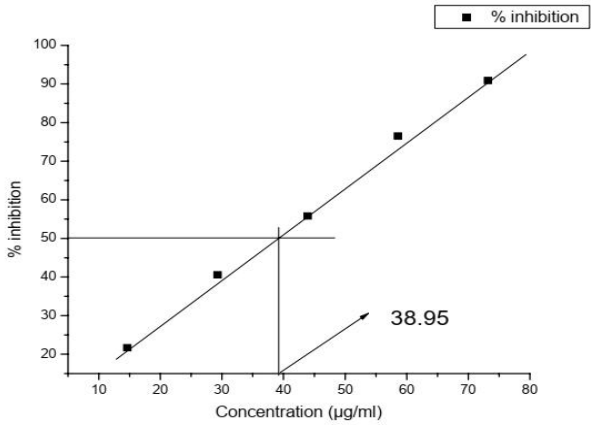

Fig.-3: Antioxidant Assay of HL^2

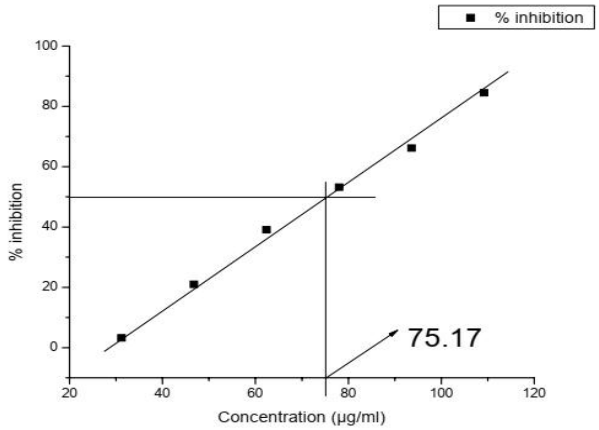

Fig.-4: Antioxidant Assay of HL^3

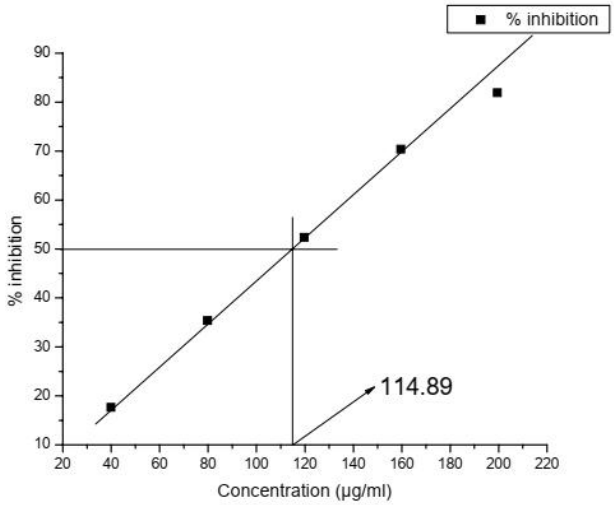

Fig.-5: Antioxidant Assay of HL^6

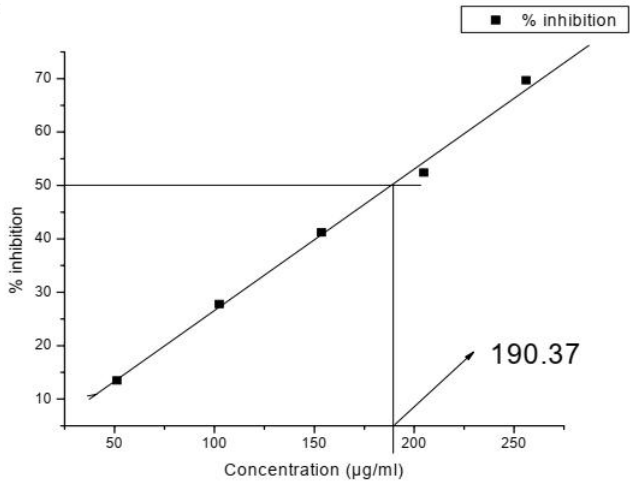

Fig.-6: Antioxidant Assay of HL^8

Table-2: Antioxidant Assay of the Cu(II) Complexes of Active Unsaturated β-ketoesters

Complex	Concentration (µg/mL)	Absorbance	% Difference	% Inhibition	EC_{50} (µg/mL)
$[CuL^2{}_2]$	0	0.698	-	-	46.37
	18.96	0.532	76.2	23.8	
	37.92	0.429	61.5	38.5	
	56.88	0.275	39.4	60.6	
	76.00	0.146	20.9	79.1	
	94.8	0.082	12.8	87.2	
$[CuL^3{}_2]$	0	0.698	-	-	85.37
	33.6	0.528	75.64	24.36	
	67.2	0.471	59.74	40.26	
	100.8	0.285	40.80	59.11	
	134.4	0.175	21.07	74.93	
	168.0	0.091	13.20	86.80	
$[CuL^6{}_2]$	0	0.698	-	-	108.16
	42.5	0.539	77.3	22.7	
	85.0	0.411	58.9	41.1	
	127.5	0.268	38.4	61.6	
	170.0	0.162	23.3	76.7	
	212.5	0.092	13.2	86.8	
$[CuL^8{}_2]$	0	0.683	-	-	171.70
	61.2	0.592	86.67	13.33	
	120.4	0.438	64.13	35.87	
	180.4	0.314	45.97	54.07	
	240.5	0.197	28.84	71.16	
	300.1	0.075	10.98	89.02	

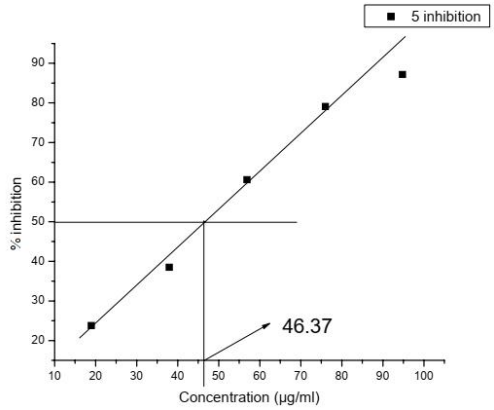

Fig.-7: Antioxidant assay of $[CuL^2{}_2]$

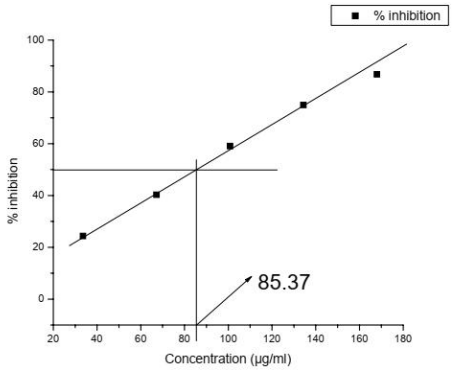

Fig.-8: Antioxidant assay of $[CuL^3{}_2]$

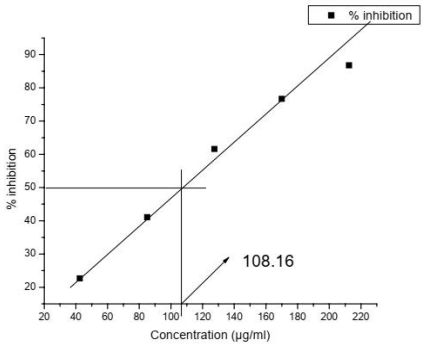

Fig.-9: Antioxidant Assay of $[CuL^6{}_2]$

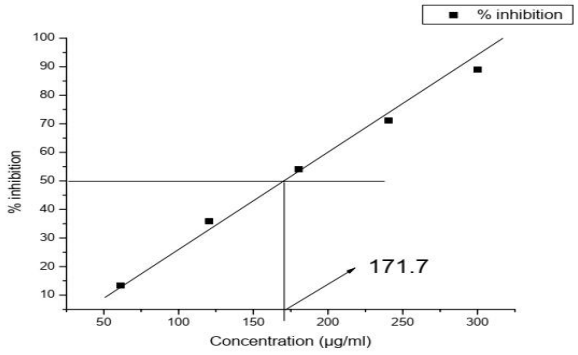

Fig.-10: Antioxidant Assay of $[CuL^8{}_2]$

Table-3: Anti-Radical Power (ARP), Stoichiometric Value (SV) and Number of Reduced DPPH (NRD) of the Active Unsaturated β-ketoesters and their Cu(II) complexes

Compound	ARP	SV	NRD
HL^2	7.40	0.28	3.57
HL^3	4.00	0.50	2.00
HL^6	2.30	0.86	1.16
HL^8	1.70	1.16	0.86
$[CuL^2_2]$	14.28	0.14	7.14
$[CuL^3_2]$	6.66	0.30	3.33
$[CuL^6_2]$	5.55	0.36	2.77
$[CuL^8_2]$	4.16	0.48	2.08

CONCLUSION

This study demonstrates that phenolic hydroxyl groups play a decisive role in enhancing the antioxidant activity of unsaturated β-ketoesters and their Cu(II) complexes. Ligands HL^2, HL^3, HL^6, and HL^8 exhibited the highest radical scavenging potential, which was further amplified upon coordination with Cu(II), due to the $[CuL_2]$ stoichiometry that enables Cu(II)/Cu(I) redox cycling. The results offer useful information on the structure–activity relationships of synthetic curcuminoid analogues and highlight the potential of β-ketoester-based metal complexes as efficient antioxidants. These results contribute to the design of novel antioxidant compounds with potential applications in pharmaceutical and biomedical fields, aligning with Sustainable Development Goal 3 (Good Health and Well-being).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided by the University Grants Commission (UGC), New Delhi.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest concerning the publication of this article.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*, All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: S. Thelakkadan and M. B. Ummathur, *Rasayan J. Chem.*, 19(1), 348-354(2026), https://doi.org/10.317 88/RJC.2026.1918560.

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