INNSPUB

**International Journal of Biosciences | IJB |**
**ISSN: 2220-6655 (Print); 2222-5234 (Online)**
**Website: https://www.innspub.net**
**Email contact: info@innspub.net**
**Vol. 29, Issue: 5, p. 1-7, 2026**

RESEARCH PAPER OPEN ACCESS

# An *in vitro* free radical scavenging efficacy of chelidonic acid

**N. Brindhadevi, K. Harish, S. M. Sivasankaran, B. Vidhyambigai, S. Manoharan***

*Department of Biochemistry and Biotechnology, Annamalai University, Chidambaram, Tamil Nadu, India*

**Key words:** Chelidonic acid, Free radical scavenging, Antioxidant, Reactive oxygen species, Oxidative stress

**Received:** October 15, 2026 **Accepted:** October 25, 2026 **Published:** November 03, 2026

**DOI:** https://dx.doi.org/10.12692/ijb/29.5.1-7

**ABSTRACT**

Oxidative stress resulting from an excess production of reactive oxygen species (ROS) is strongly linked to cell damage and development of various chronic disorders. This study investigates the free radical scavenging activity of chelidonic acid using various colorimetric assays. The free radical scavenging activity of chelidonic acid was tested at various concentrations (20, 40, 60, 80 and 100 µg/mL) by utilizing the DPPH, $ABTS^+$, hydroxyl radical, superoxide radical scavenging and hydrogen peroxide ($H_2O_2$) decomposition as well as reducing power assay. Ascorbic acid and trolox served as the reference control for these assays. Chelidonic acid exhibited dose dependent free radical scavenging activity across all assays. The DPPH (27.79 µg/mL) and $ABTS^+$ (59.20 µg/mL) scavenging assays exhibited near $IC_{50}$ values compared to reference compounds ascorbic acid (25.55 µg/mL) and Trolox (54.84 µg/mL), respectively. Chelidonic acid showed greater hydroxyl radical scavenging activity than the standard, with an $IC_{50}$ value of 30.77 µg/mL compared with 50.47 µg/mL noticed for ascorbic acid. Chelidonic acid also showed potent superoxide radical scavenging activity, with an $IC_{50}$ value of 27.73 µg/mL compared to 36.36 µg/mL for ascorbic acid. $H_2O_2$ scavenging activity showed $IC_{50}$ values of 25.20 µg/mL for chelidonic acid and 26.16 µg/mL for ascorbic acid. In the ferric reducing assay (FRAP), chelidonic acid exhibited substantial reducing capacity, with an $IC_{50}$ value of 48.36 µg/mL compared with 43.87 µg/mL for ascorbic acid. The free radical scavenging activity of chelidonic acid suggests its ability to attenuate the oxidative stress induced by ROS.

***Corresponding author:** S. Manoharan ✉ sakshiman@rediffmail.com
https://orcid.org/0000-0001-5233-9071

## INTRODUCTION

Free radicals are highly reactive molecules having one or more unpaired electrons, making them chemically unstable (Chandimali *et al.,* 2025). These radicals comprise of ROS and reactive nitrogen species (RNS). They are generated in the living systems during biological and metabolic processes due to internal and external stimuli. One of the primary sources of free radicals within the body includes the electron transport chain (ETC) of mitochondria, which can produce superoxide anions ($O_2^-$) via oxidative phosphorylation (Di Meo *et al.*, 2016). Free radicals can also arise during inflammatory processes and enzymatic reactions as well as following exposure to environmental stressors such as pollutants, radiation and xenobiotics (Pooja *et al.,* 2025).

Under physiological conditions, free radicals play essential roles in cellular signaling, immune defense and homeostasis. However, excessive production of ROS and RNS or inadequate antioxidant defense system results in oxidative stress leads to damage to cellular macromolecules such as lipids, proteins and DNA (Liu *et al.,* 2025) as well as its implicated in the pathogenesis of several chronic diseases, including cancer, cardiovascular disorders, diabetes mellitus and neurodegenerative diseases (Dash *et al.,* 2025). In cancer, chronic oxidative stress contributes to DNA mutations, genomic instability and altered cell signaling and tumor progression, thereby playing a critical role in cancer initiation, promotion and metastasis (Feng *et al.,* 2026).

*In vitro* antioxidant assays provide a reliable and widely accepted approach to determine the free radical scavenging ability of natural compounds. Medicinal plants have long been recognized as rich sources of bioactive compounds with significant antioxidant efficacy. Plant derived antioxidants can neutralize free radicals, reduce oxidative stress and thereby protecting oxidative stress associated diseases. Several studies have been reported that phytochemicals exhibit antioxidant, antiinflammatory and anticancer activities, making them attractive candidates for chemopreventive and therapeutic applications (Chandimali *et al.*, 2025; Tüğen *et al.,* 2025).

Chelidonic acid (4-oxopyran-2,6-dicarboxylic acid) (Fig. 1) is a naturally occurring compound present in certain medicinal plants including *Chelidonium majus, Sorghum vulgare, Cassia spectabilis* and *Gloriosa superba*. In the last few years, chelidonic acid has gained much attention due to its multiple pharmacological activities. Experimental studies reported that chelidonic acid exhibited neuroprotective, cardioprotective, nephroprotective, antiallergic and immunomodulatory activities. These activities of chelidonic acid are mainly due to its antioxidant, antiinflammatory and apoptotic efficacy (Harish *et al.,* 2024).

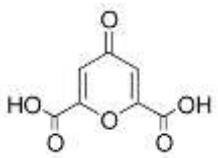



**Fig. 1.** Molecular Structure of chelidonic acid

The present investigation aims to explore the free radical scavenging activities of chelidonic acid through various assays such as DPPH assay, $ABTS^+$, hydroxyl radical, superoxide radical, hydrogen peroxide scavenging and reducing power assay.

## MATERIALS AND METHODS

### Chemicals

Chelidonic acid was procured from Sigma-Aldric, Bangalore, India. All other reagents used in the study were of analytical grade.

### *In Vitro* free radical scavenging assays

*DPPH radical scavenging assay*

The DPPH radical scavenging activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by Villaño *et al.* (2007). Different concentrations of chelidonic acid and ascorbic acid (20-100 µg/mL) were added to methanolic DPPH solution, vortex and incubated at room temperature. The decrease in absorbance was measured spectrophotometrically at 517 nm against a control lacking the test sample.

*$ABTS^+$ radical scavenging assay*

The ABTS radical cation ($ABTS^+$) scavenging activity was determined according to Rumpf *et al.* (2023). The reaction mixture containing ABTS, hydrogen peroxide ($H_2O_2$) and sodium phosphate buffer (pH 7.5) were treated with chelidonic acid (20-100 µg/mL). Absorbance was recorded at 734 nm and compared with trolox.

*Hydroxyl radical scavenging assay*

Hydroxyl radical scavenging activity was assessed by deoxyribose degradation method described by Halliwell *et al.* (1987). The reaction mixture contained 2-deoxyribose in potassium dihydrogen phosphate potassium hydroxide buffer ($KH_2PO_4$–KOH, pH 7.4), EDTA-$FeCl_3$, $H_2O_2$ and chelidonic acid (20-100 µg/mL) was incubated at 37 °C for 1 h. After reaction with thiobarbituric acid (TBA) and trichloroacetic acid (TCA) and heating, absorbance was measured at 532 nm. Ascorbic acid used as the standard drug.

*Superoxide radical scavenging assay*

Nishikimi *et al.* (1972) method was adopted for determining the ability of chelidonic acid to scavenge superoxide radicals. This method involves the coupling reaction between phenazinemethosulfate and nicotinamide adenine dinucleotide, leading to the production of superoxide radicals that reduce nitroblue tetrazolium into formazan, which appears as a purple colored complex. Formazan was determined through a spectrophotometric reading at 560 nm wavelength.

*Hydrogen peroxide ($H_2O_2$) scavenging assay*

The hydrogen peroxide scavenging activity was evaluated following the method of Jayaprakasha *et al.* (2004). Chelidonic acid (20-100 µg/mL) was mixed with $H_2O_2$ prepared in phosphate buffered saline (PBS, pH 7.4), incubated for 10 min and absorbance was recorded at 230 nm.

*Reducing power assay*

The reducing power of chelidonic acid was determined according to Benzie and Strain., (1996). Samples were mixed with phosphate buffer and potassium ferricyanide, incubated at 50 °C for 20 min. It was there treated with TCA, centrifuged and treated with ferric chloride. Absorbance was measured at 700 nm.

**Calculation of scavenging activity**

For all assays, the percentage of scavenging activity was calculated using the following equation:

$$\%\text{Scavenging Activity} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100$$

The $IC_{50}$ value was defined as the concentration of chelidonic acid required to scavenge 50% of free radicals. All the assays were performed in triplicate.

**Statistical analysis**

The results are expressed as mean ± standard deviation (SD). Differences among groups (n=3) at each concentration were analyzed using Student's t-test, with $p < 0.05$ considered statistically significant. Graphs were prepared using GraphPad Prism.

## RESULTS AND DISCUSSION

### Free radical scavenging activity of chelidonic acid

Oxidative stress involves the excessive generation of ROS, including free radicals and reactive oxidants, which can cause oxidative damage to cellular macromolecules (Pizzino *et al.*, 2017). Since each ROS differ in their chemical reactivity and biological effects, a single antioxidant assay alone cannot fully reveal the antioxidant potential of a test compound.

Therefore, the free radical scavenging activity of chelidonic acid was evaluated using six *in vitro* assays, namely DPPH, $ABTS^+$, hydroxyl radical, superoxide radical, hydrogen peroxide scavenging and reducing power assays. Together, these assays assess different antioxidant mechanisms, including hydrogen atom or electron donation, direct radical neutralization, elimination of ROS and reduction of oxidized metal ions.

The DPPH radical scavenging assay is widely used to evaluate the hydrogen or electron donating ability of

test compound towards the formation of stable DPPH radical. Reduction of DPPH results in a decrease in absorbance, with greater reduction reflecting stronger radical scavenging activity (Baliyan *et al.,* 2022). Chelidonic acid exhibited a concentration dependent increase in DPPH radical scavenging activity, with an $IC_{50}$ value of 27.79 µg/mL compared with 25.55 µg/mL for ascorbic acid (Fig. 2). The relatively close $IC_{50}$ values indicate that chelidonic acid possesses favorable DPPH radical quenching capacity. This activity may be related to the ability of its functional groups to participate in hydrogen atom or electron transfer reactions, thereby stabilizing the reactive radical species.

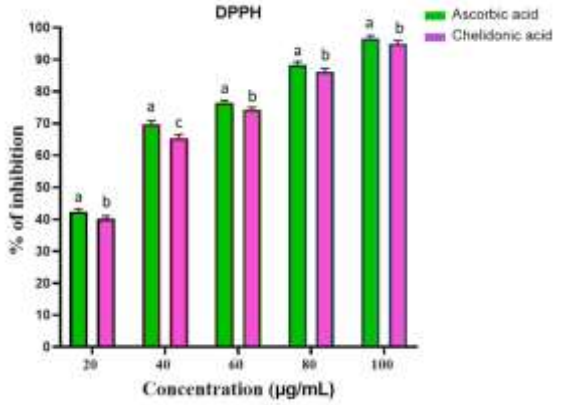



**Fig. 2.** DPPH radical scavenging ability of chelidonic acid and ascorbic acid

Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.02; cp< 0.001).

The $ABTS^+$ radical scavenging assay provides complementary information on test compound activity by measuring the efficacy of a compounds to neutralize the blue green $ABTS^+$ radical cation through electron or hydrogen transfer (Sivasankaran *et al.,* 2023). Chelidonic acid showed a dose dependent increase in $ABTS^+$ scavenging activity with an $IC_{50}$ value of 59.20 µg/mL compared with 54.84 µg/mL for trolox (Fig. 3). The relatively near $IC_{50}$ values demonstrate favorable reaction of chelidonic acid with the $ABTS^+$ radical cation. The consistent activity observed in both DPPH and $ABTS^+$ assays suggesting that the antioxidant potential of chelidonic acid is not limited to a single radical system.

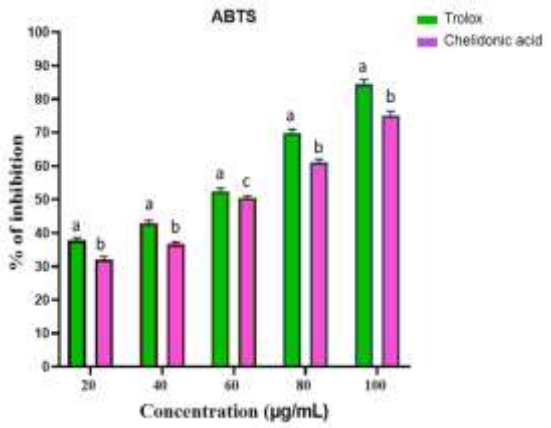



**Fig. 3.** $ABTS^+$ radical scavenging ability of chelidonic acid and trolox

Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.001; cp< 0.02).

The hydroxyl radical scavenging assay determines the ability of test compounds to protect against hydroxyl radicals, which are among the most reactive ROS and can directly affect the lipids, proteins and nucleic acids (Lalhminghlui *et al.,* 2018). Chelidonic acid demonstrated pronounced hydroxyl radical scavenging activity with an $IC_{50}$ value of 30.77 µg/mL compared with 50.47 µg/mL for ascorbic acid (Fig. 4). The lower $IC_{50}$ value indicates greater hydroxyl radical scavenging efficiency of chelidonic acid. This strong activity suggesting that chelidonic acid may effectively intercept highly reactive hydroxyl radicals and thereby limit their contribution to oxidative molecular damage.

The superoxide radical scavenging assay mearsures the ability of test compounds to neutralize superoxide anions, which can contribute to the formation of secondary ROS such as $H_2O_2$ and hydroxyl radicals (Gulcin., 2025). Chelidonic acid exhibited a dose dependent increase in $O_2^-$ inhibition with an $IC_{50}$ value of 27.73 µg/mL compared with 36.36 µg/mL for ascorbic acid (Fig. 5). The lower $IC_{50}$ value indicates greater superoxide radical scavenging efficiency of chelidonic acid than the ascorbic acid. This pronounced activity may help mitigates ROS progression and contribute to the maintenance of cellular redox homeostasis.

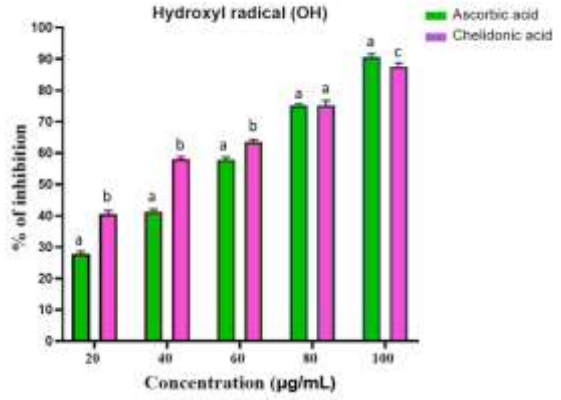



**Fig. 4.** Hydroxyl radical scavenging ability of chelidonic acid and ascorbic acid
Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.001; cp< 0.02).

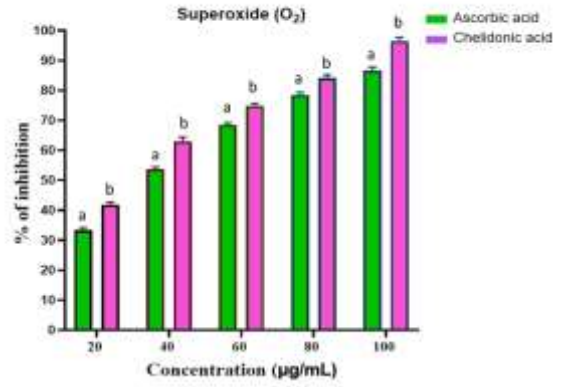



**Fig. 5.** Superoxide anion scavenging ability of chelidonic acid and ascorbic acid
Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.001).

The hydrogen peroxide ($H_2O_2$) scavenging assay measures the ability of antioxidants to remove $H_2O_2$, an important non radical ROS that can participate in metal catalyzed reactions leading to highly reactive hydroxyl radicals (Rampon *et al.,* 2023). Chelidonic acid showed concentration dependent $H_2O_2$ scavenging activity with an $IC_{50}$ value of 25.20 µg/mL compared with 26.16 µg/mL for ascorbic acid (Fig. 6). The slightly lower $IC_{50}$ value indicates marginally greater $H_2O_2$ scavenging activity of chelidonic acid than the standard.

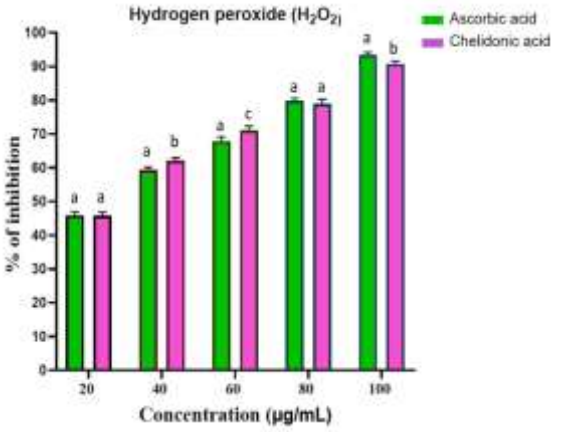



**Fig. 6.** Hydrogen peroxide radical scavenging ability of chelidonic acid and ascorbic acid
Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.01; cp< 0.02).

Effective $H_2O_2$ removal may therefore contribute to limiting reduced ROS formation and subsequent oxidative chain reactions.

The reducing power assay determines the electron donating capalities of test compounds based on their ability to reduce ferric ions to ferrous ions. Increased absorbance reflects higher reducing capacity (Spiegel *et al.,* 2020). Chelidonic acid demonstrated a dose dependent increase in ferric reducing activity with an $IC_{50}$ value of 48.36 µg/mL compared with 43.87 µg/mL of ascorbic acid (Fig. 7). Although the reducing capacity of chelidonic acid was slightly lower than that of ascorbic acid, the observed activity indicates that the compound exhibited electron donating potential, which may contribute to its antioxidant action.

The results obtained from these six antioxidant assays shown that chelidonic acid has multifunctional free radical scavenging potential. The strong scavenging activities toward hydroxyl radicals and $H_2O_2$ accompanied by favorable scavenging activity of DPPH, $ABTS^+$, superoxide radicals and ferric reducing activity, indicate that its antioxidant effect is not dependent on a single reaction mechanism. Notably, the capacity of chelidonic acid to scavenge various ROS and reactive radicals is biologically relevant since the

oxidative stress condition is marked by the simultaneous generation and interaction of many reactive species.

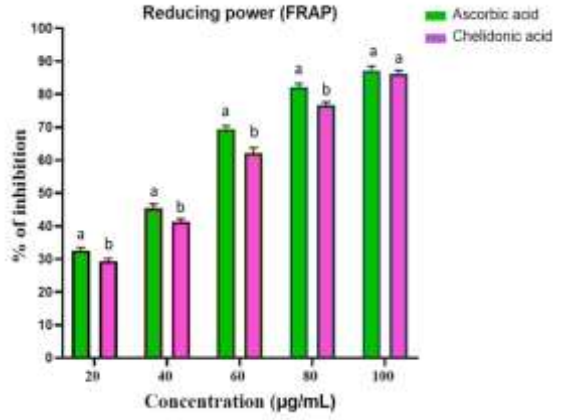



**Fig. 7.** Reducing power ability of chelidonic acid and ascorbic acid

Values are expressed as mean ± S.D. (n = 3). Different superscript letters indicate significant differences (bp < 0.001).

The inhibition of ROS accumulation by donation of electrons or hydrogen atoms to reactive species may contribute to reducing oxidative stress cascades as well as and maintaining redox balance. The obtained results serve as experimental evidence of antioxidant properties of chelidonic acid and a basis of its protective activity against the cellular damage linked to oxidative stress.

## CONCLUSION

The present study demonstrates that chelidonic acid exhibited broad spectrum of *in vitro* free radical scavenging and efficacy through complementary radical neutralizing, electron donating and reducing mechanisms. Chelidonic acid showed particularly strong scavenging activity against hydroxyl and superoxide radicals, with slightly greater $H_2O_2$ scavenging activity than ascorbic acid, while DPPH and $ABTS^+$ activities were comparable to the standard. The observed free radical scavenging activity may be associated with the chemical structure of chelidonic acid, particularly its 4-oxopyran ring and two carboxylic acid groups, which provide functional sites capable of participating in electron transfer and radical interaction processes. Through these chemical properties, chelidonic acid may effectively attenuate ROS and limit the progression of oxidative reactions. This findings revealed chelidonic acid as a promising natural antioxidant with spectrum of free radical scavenging potential and provide a basis for further investigation of its protective effect against oxidative stress associated cellular damage in *in vitro* experimental animal models.

## ACKNOWLEDGEMENTS

Financial assistance from Tamil Nadu Government to N. Brindhadevi in the form of SC/ST Ph.D. Scholars' Scholarship Scheme is gratefully acknowledged.

## REFERENCES

**Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM.** 2022. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules **27**, 1326.

**Benzie IF, Strain JJ.** 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Analytical Biochemistry **239**, 70-76.

**Chandimali N, Bak SG, Park EH, Lim HJ, Won YS, Kim EK, Park SI, Lee SJ.** 2025. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discovery **11**, 19.

**Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, Panda SK, Kerry RG, Duttaroy AK, Jena AB.** 2025. Oxidative stress and inflammation in the pathogenesis of neurological disorders: Mechanisms and implications. Acta Pharmaceutica Sinica B **15**, 15-34.

**Di Meo S, Reed TT, Venditti P, Victor VM.** 2016. Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxidative Medicine and Cellular Longevity **2016**, 1245049.

**Feng X, Liang G, Sun J, Wang Z, Li T, Fu Y, Deng W, Li X, Luo J, Jin P, Ge C, Li R.** 2026. Impact of oxidative stress on malignant tumor progression and emerging therapeutic strategies. Frontiers in Molecular Biosciences **13**, 1916698.

**Gulcin İ.** 2025. Antioxidants: a comprehensive review. Archives of Toxicology **99**, 1893-1997.

**Halliwell B, Gutteridge JM, Aruoma OI.** 1987. The deoxyribose method: a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry **165**, 215-219.

**Harish K, Sivasankaran SM, Manoharan S.** 2024. Indian Medicinal Plants with Multiple Pharmacological Efficacies: A Comprehensive Review. Journal of Drug Delivery and Therapeutics **14**, 143-154.

**Jayaprakasha GK, Jaganmohan Rao L, Sakariah KK.** 2004. Antioxidant activities of flavidin in different in vitro model systems. Bioorganic and Medicinal Chemistry **12**, 5141-5146.

**Lalhminghlui K, Jagetia GC.** 2018. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, *Schima wallichii* Korth *in vitro*. Future Science OA **4,** FSO272.

**Liu S, Liu J, Wang Y, Deng F, Deng Z.** 2025. Oxidative Stress: Signaling Pathways, Biological Functions, and Disease. MedComm **6,** e70268.

**Nishikimi M, Appaji N, Yagi K.** 1972. The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochemical and Biophysical Research Communications **46**, 849-854.

**Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A.** 2017. Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity **2017**, 8416763.

**Pooja G, Shweta S, Patel P.** 2025. Oxidative stress and free radicals in disease pathogenesis: a review. Discovery Medicine **2**, 104.

**Rampon C, Vriz S.** 2023. Hydrogen Peroxide Signaling in Physiology and Pathology. Antioxidants **12**, 661.

**Rumpf J, Burger R, Schulze M.** 2023. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. International Journal of Biological Macromolecules **233**, 123470.

**Sivasankaran SM, Abdulla SH, Elanchezhiyan C, Pethanasamy M, Surya S, Theerthu A, Krishnan H.** 2023. Reactive Oxygen Species Scavenging and Anti-Proliferative Potential of Veratric Acid: An in vitro Approach. Tropical Journal of Natural Product Research **7**, 2837.

**Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Żbikowska B, Hill GA, Sroka Z.** 2020. Antioxidant Activity of Selected Phenolic Acids-Ferric Reducing Antioxidant Power Assay and QSAR Analysis of the Structural Features. Molecules **25**, 3088.

**Tüğen A, Buruleanu CL.** 2025. The Role of Plant-Derived Bioactive Compounds in Mitigating Oxidative Stress. Foods **15**, 108.

**Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM, García-Parrilla MC.** 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta **11**, 230-235.