

Exploring the Antioxidant Properties of *Digera muricata* (L.) Mart. Leaves Through *In Vitro* Assays

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Received: 8th June, 2026; **Revised:** 20th June, 2026; **Accepted:** 30th June, 2026; **Available Online:** 10th July, 2026

ABSTRACT

The present study evaluated the *in vitro* antioxidant activity of the hydro-ethanolic leaf extract of *Digera muricata* using five different antioxidant assays, namely DPPH radical scavenging, nitric oxide radical scavenging, ion chelating, hydroxyl radical scavenging, and hydrogen peroxide scavenging activities. The antioxidant potential was assessed at concentrations ranging from 20–100 µg/ml. The extract exhibited a concentration-dependent increase in antioxidant activity in all assays. In the DPPH assay, the percentage inhibition increased from $18.07 \pm 1.35\%$ to $85.68 \pm 1.23\%$, with an IC_{50} value of 52.0 µg/ml. Nitric oxide scavenging activity ranged from $17.53 \pm 1.52\%$ to $85.60 \pm 1.38\%$, with an IC_{50} of 53.1 µg/ml. The ion chelating assay demonstrated inhibition values between $16.79 \pm 1.32\%$ and $88.22 \pm 1.15\%$, yielding an IC_{50} value of 54.8 µg/ml. Hydroxyl radical scavenging activity increased from $19.55 \pm 1.30\%$ to $87.70 \pm 1.29\%$, with an IC_{50} of 56.0 µg/ml. Similarly, hydrogen peroxide scavenging activity ranged from $15.25 \pm 1.25\%$ to $88.34 \pm 1.35\%$, with an IC_{50} value of 53.5 µg/ml. The results indicate that the extract possesses significant free radical scavenging and metal chelating properties, which may be attributed to the presence of phenolic compounds, flavonoids, and other bioactive phytochemicals. The findings suggest that *Digera muricata* leaves are a promising natural source of antioxidants and could be utilized for the development of therapeutic agents against oxidative stress-related disorders.

Keywords: *Digera muricata*, Antioxidant activity, DPPH radical scavenging, Free radical inhibition, Ion chelating activity, Phytochemicals

How to cite this article: Tamarasan S, Vidya R. Exploring the Antioxidant Properties of *Digera muricata* (L.) Mart. Leaves Through *In Vitro* Assays. Int J Drug Deliv Technol. 2026;16(68s):89-95. DOI: 10.25258/ijddt.16.68s.9

INTRODUCTION

Digera muricata (L.) Mart., commonly known as Tartara or false amaranth, is an annual herb belonging to the family Amaranthaceae. It is widely distributed throughout tropical and subtropical regions of Asia and Africa, particularly in India, where it grows as a common weed in agricultural fields and wastelands. The plant is traditionally consumed as a leafy vegetable and has been used in folk medicine for the treatment of various ailments, including diabetes, inflammation, urinary disorders, digestive problems, and skin diseases (Sangeetha Ravi et al., 2023). Recent scientific investigations have revealed that *D. muricata* is rich in biologically active phytochemicals such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, terpenoids, β -sitosterol, quercetin, and other antioxidant constituents. These compounds contribute to its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and anti-atherosclerotic

properties (Anita., 2024). Due to its nutritional value and therapeutic potential, *Digera muricata* has attracted increasing attention from researchers as a promising source of natural bioactive compounds for pharmaceutical and nutraceutical applications. Several studies have demonstrated its ability to scavenge free radicals, inhibit microbial growth, and exhibit cytotoxic effects against cancer cell lines, highlighting its potential role in the development of novel plant-based medicines (Podhigai Selvi Varadarajan et al., 2023).

Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, plays a major role in the development of several chronic diseases such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Natural antioxidants derived from medicinal plants have attracted considerable attention because they can neutralize free radicals and protect biological systems from oxidative damage (Sharma P et al., 2025). Several *in vitro* studies have demonstrated

significant antioxidant activity in leaf extracts of *D. muricata* using assays such as DPPH radical scavenging, superoxide radical scavenging, hydroxyl radical scavenging, metal chelation, and reducing power assays. Among different solvent extracts, the methanolic extract exhibited the highest antioxidant potential, which was strongly correlated with its elevated total phenolic content. These findings suggest that phenolic compounds play a major role in the free radical scavenging activity of the plant and support its potential use as a natural antioxidant for preventing oxidative stress-mediated disorders (Abdul Rahim et al., 2025).

MATERIALS AND METHOD

Preparation of samples

The leaves of *Digera muricata* were dried in shade, powdered coarsely and macerated in cold under the conditions of hydro-ethanolic solvent (ethanol:distilled water, 70:30 v/v) over 24 h with intervals of shaking. The resultant slurry was filtered through Whatman No. 1 filter paper, and used for the *in vitro* antioxidant assays. Briefly, the dried crude extract was accurately weighed and dissolved in analytical-grade methanol to prepare a stock solution of 1 mg/mL. The stock solution was vortexed thoroughly until complete dissolution and filtered through a 0.22 µm membrane filter to remove any insoluble particles. Working solutions of 20, 40, 60, 80, and 100 µg/mL were freshly prepared by serial dilution of the stock solution using methanol immediately before each experiment.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992). Briefly, a 2 ml aliquot of DPPH methanol solution (25 µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = control is the absorbance and A_s = sample is the absorbance of reaction mixture (in the presence of sample).

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. About 2 ml of 10 mM sodium nitroprusside

in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide scavenging activity was calculated according to the following equation: % Inhibition = $((A_0 - A_1) / A_0 \times 100)$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Fe²⁺ chelating activity assay

The metal chelating activity of the extract was assayed by Dinis *et al.*, (1994) method. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of FeCl₂ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 minutes at room temperature, the absorbance of the Fe²⁺-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe²⁺ was calculated as: Chelating rate (%) = $[\text{Absorbance of control} - \text{Absorbance of sample} / \text{Absorbance of control}] \times 100$

Hydrogen Peroxide scavenging activity

Hydrogen peroxide scavenging activity is performed according to the methodology of Ruch *et al.*, 1989, with slight modifications. 1 ml of test sample (20 to 100 µg/ml) was taken in different test tubes to which 1 ml of H₂O₂ was added. The tubes were incubated for 5 minutes at room temperature. After 5 mins, 2 ml of potassium dichromate: acetic acid reagent was added and the tubes were incubated for 10 minutes at room temperature. The absorbance value of the reaction mixture was recorded at 700 nm. Blank containing the phosphate buffer without the plant extract.

$$\% \text{ Scavenging } [H_2O_2] = [A_{\text{control}} - A_{\text{test}} / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance of the control, and A_{test} is the absorbance of the sample.

Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al.* (2004). The hydrogen peroxide was added to the reaction mixture containing 60 µl of 1.0 mM FeCl₃, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H₂O₂, and 1.5 ml of extract at various concentrations. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity was

calculated according to the following equation: % Inhibition = $((A_0 - A_1) / A_0 \times 100)$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Fe²⁺ chelating activity assay

The metal chelating activity of the extract was assayed by Ruch *et al.*, (1989) method. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of FeCl₂ (2mM) was added. After 30 s, 0.1 ml ferrozine (5mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 minutes at room temperature, the absorbance of the Fe²⁺-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe²⁺ was calculated as: Chelating rate (%) = [Absorbance of control – Absorbance of sample / Absorbance of control] x 100

Statistical analysis

Tests were carried out in triplicates. The amount of sample needed to inhibit free radicals by 50%, IC₅₀, was graphically determined by a linear regression method using MS- Windows based graphpad Instat (version 3) software. Results were expressed as mean ± standard deviation.

RESULTS

DPPH radical scavenging activity

The DPPH radical scavenging assay was used to evaluate the antioxidant potential of the hydro-ethanolic leaf extract of *Digera muricata*. The extract exhibited a concentration-dependent increase in free radical scavenging activity. At a concentration of 20 µg/ml, the extract showed 18.07 ± 1.35% inhibition of DPPH radicals. As the concentration increased to 40 µg/ml, the percentage inhibition increased to 36.91 ± 1.27%. A further increase in concentration resulted in enhanced antioxidant activity, with 58.56 ± 1.44% inhibition observed at 60 µg/ml. At concentrations of 80 and 100 µg/ml, the extract demonstrated 72.69 ± 1.30% and 85.68 ± 1.23% inhibition, respectively (Table 1 & Figure 1). The progressive increase in radical scavenging activity with increasing concentration indicates the presence of bioactive compounds capable of donating hydrogen atoms or electrons to neutralize DPPH free radicals. The IC₅₀ value of the extract was found to be 52.0 µg/ml, indicating that a relatively low concentration of the extract was sufficient to scavenge 50% of DPPH radicals. Lower IC₅₀ values generally reflect stronger antioxidant activity. The significant antioxidant potential observed in the extract may be attributed to the presence of phenolic compounds, flavonoids, tannins, and other phytoconstituents known for their free radical scavenging properties.

Nitric oxide scavenging activity

The nitric oxide radical scavenging assay was performed to assess the antioxidant potential of the hydro-ethanolic leaf extract of *Digera muricata*. The extract exhibited a concentration-dependent increase in nitric oxide scavenging activity. At a concentration of 20 µg/ml, the extract showed 17.53 ± 1.52% inhibition of nitric oxide radicals. The percentage inhibition increased to 34.55 ± 1.30% at 40 µg/ml, indicating an enhanced radical scavenging effect with increasing concentration. At 60 µg/ml, the extract demonstrated 58.11 ± 1.25% inhibition, exceeding the 50% scavenging threshold. Further increases in concentration to 80 and 100 µg/ml resulted in 73.82 ± 1.49% and 85.60 ± 1.38% inhibition, respectively (Figure 1). These results indicate that the extract possesses a strong ability to neutralize nitric oxide radicals, thereby reducing the formation of reactive nitrogen species that contribute to oxidative stress and cellular damage. The IC₅₀ value of the extract was found to be 53.1 µg/ml, suggesting effective nitric oxide scavenging activity at a relatively low concentration (Table 1). The antioxidant effect may be attributed to the presence of phenolic compounds, flavonoids, alkaloids, and other phytochemicals capable of donating electrons to stabilize free radicals. The concentration-dependent increase in inhibition demonstrates the efficiency of the extract in preventing nitric oxide-mediated oxidative damage. These findings suggest that *Digera muricata* leaf extract possesses significant nitric oxide radical scavenging activity and may serve as a potential natural antioxidant source for protecting biological systems against oxidative and nitrosative stress. The observed activity is comparable to that reported for many medicinal plants rich in phenolic and flavonoid constituents.

Table 1: Antioxidant activity of hydroethanolic extract of *Digera muricata*

Concentration (µg/ml)	In vitro antioxidant assays				
	DPPH radical scavenging activity	Nitric oxide radical scavenging activity	Fe ²⁺ chelating activity	Hydroxyl radical scavenging activity	Hydrogen peroxide scavenging activity

20	18.07 ±1.3 5	17.53 ±1.5 2	16.7 9±1. 32	19.55 ±1.3 0	15.25 ±1.2 5
40	36.91 ±1.2 7	34.55 ±1.3 0	37.3 6±1. 25	33.51 ±1.2 5	34.32 ±1.3 5
60	58.56 ±1.4 4	58.11 ±1.2 5	54.4 7±1. 38	54.07 ±1.2 7	57.62 ±1.2 5
80	72.69 ±1.3 0	73.82 ±1.4 9	70.6 4±1. 35	73.18 ±1.3 0	70.33 ±1.3 0
100	85.68 ±1.2 3	85.60 ±1.3 8	88.2 2±1. 15	87.70 ±1.2 9	88.34 ±1.3 5
IC₅₀ (µg/ml)	52.0	53.1	54.8	56.0	53.5

Value expressed as mean ± SD (n=3)

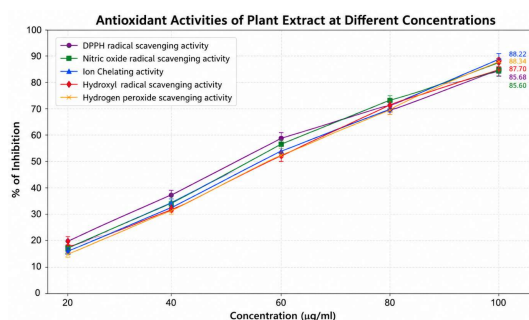


Figure 1: Antioxidant activity of Hydroethanolic extract of *Digera muricata*

Iron chelating activity

The ion chelating assay was carried out to evaluate the metal chelating potential of the hydro-ethanolic leaf extract of *Digera muricata*. The extract demonstrated a concentration-dependent increase in ferrous ion chelating activity. At a concentration of 20 µg/ml, the extract exhibited 16.79 ± 1.32% chelation of ferrous ions. The chelating activity increased to 37.36 ± 1.25% at 40 µg/ml, indicating enhanced metal-binding capacity with increasing concentration. At 60 µg/ml, the extract showed 54.47 ± 1.38% chelating activity, suggesting that more than half of the ferrous ions present were effectively bound by the phytochemicals in the extract. Further increases in concentration to 80 and 100 µg/ml resulted in 70.64 ± 1.35% and 88.22 ± 1.15% chelation, respectively (Figure 1). The

highest concentration tested exhibited the strongest metal chelating effect, demonstrating the extract's ability to inhibit metal-catalyzed oxidative reactions. The IC₅₀ value was determined to be 54.8 µg/ml, indicating that a moderate concentration of the extract was sufficient to chelate 50% of the ferrous ions (Table 1).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay was performed to evaluate the ability of the hydro-ethanolic leaf extract of *Digera muricata* to neutralize hydroxyl radicals, one of the most reactive oxygen species responsible for oxidative damage to biomolecules. The extract exhibited a concentration-dependent increase in hydroxyl radical scavenging activity. At a concentration of 20 µg/ml, the extract showed 19.55 ± 1.30% inhibition of hydroxyl radicals. The scavenging activity increased to 33.51 ± 1.25% at 40 µg/ml, indicating enhanced antioxidant effectiveness with increasing concentration. At 60 µg/ml, the extract demonstrated 54.07 ± 1.27% inhibition, suggesting a substantial capacity to neutralize hydroxyl radicals. Further increases in concentration to 80 and 100 µg/ml resulted in 73.18 ± 1.30% and 87.70 ± 1.29% inhibition, respectively (Table 1 & Figure 1). The highest concentration tested exhibited the maximum scavenging activity, highlighting the strong antioxidant potential of the extract. The IC₅₀ value was found to be 56.0 µg/ml, indicating that the extract effectively scavenged 50% of hydroxyl radicals at a relatively low concentration (Table 1).

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was conducted to evaluate the antioxidant potential of the hydro-ethanolic leaf extract of *Digera muricata* against hydrogen peroxide (H₂O₂), a reactive oxygen species that can generate highly toxic hydroxyl radicals in biological systems. The extract exhibited a concentration-dependent increase in hydrogen peroxide scavenging activity. At a concentration of 20 µg/ml, the extract showed 15.25 ± 1.25% inhibition, indicating a moderate ability to neutralize hydrogen peroxide. As the concentration increased to 40 µg/ml, the scavenging activity significantly increased to 34.32 ± 1.35%. At 60 µg/ml, the extract demonstrated 57.62 ± 1.25% inhibition, exceeding the 50% scavenging level and indicating strong antioxidant potential. Further increases in concentration to 80 and 100 µg/ml resulted in 70.33 ± 1.30% and 88.34 ± 1.35% inhibition, respectively (Table 1 & Figure 1). The highest concentration tested exhibited the maximum scavenging activity, demonstrating the effectiveness of the extract in eliminating hydrogen peroxide. The IC₅₀ value of the extract was found to be 53.5 µg/ml, suggesting that a relatively low concentration was sufficient to scavenge 50% of hydrogen peroxide radicals.

DISCUSSION

The hydro-ethanolic leaf extract exhibited significant antioxidant activity in all the in vitro assays evaluated, including DPPH radical scavenging, nitric oxide scavenging, iron chelating, hydroxyl radical scavenging, and hydrogen peroxide scavenging activities. The antioxidant activity increased progressively with increasing concentration from 20 to 100 µg/ml, indicating a clear dose-dependent response. This activity can be attributed to the presence of bioactive phytochemicals such as phenolic compounds, flavonoids, tannins, alkaloids, and other secondary metabolites present in the extract, which are known to neutralize free radicals and inhibit oxidative damage.

In the DPPH radical scavenging assay, the hydro-ethanolic extract showed 85.68% inhibition at 100 µg/ml with an IC_{50} value of 52.0 µg/ml. DPPH is a stable free radical commonly used to evaluate the hydrogen-donating ability of antioxidants. The observed activity suggests that the extract contains compounds capable of donating electrons or hydrogen atoms to stabilize free radicals. Similar findings were reported by Saini (2023), who demonstrated strong DPPH radical scavenging activity in *Digera muricata* leaves, attributing the activity to their high phenolic and flavonoid content.

The nitric oxide scavenging assay revealed that the extract exhibited 85.60% inhibition at 100 µg/ml with an IC_{50} value of 53.1 µg/ml. Nitric oxide is a reactive nitrogen species that can react with oxygen to form harmful intermediates causing cellular damage. The ability of the extract to scavenge nitric oxide radicals indicates its potential to reduce oxidative stress and inflammatory responses. This finding is consistent with previous studies on medicinal plants rich in phenolic compounds, which effectively inhibit nitric oxide generation and related oxidative processes (Sharma et al., 2025). Similar antioxidant effects have been reported in phenolic-rich medicinal plant extracts, where flavonoids effectively scavenge reactive nitrogen species and prevent cellular damage (Kumar and Pandey, 2013).

The iron chelating activity of the extract reached 88.22% inhibition at 100 µg/ml with an IC_{50} value of 54.8 µg/ml. Iron chelation is an important antioxidant mechanism because iron ions catalyze the formation of highly reactive hydroxyl radicals through Fenton reactions. The significant metal-chelating ability observed in the extract suggests that its phytochemical constituents can bind transition metals, thereby preventing radical generation and oxidative damage (Rice-Evans et al., 1997). Metal chelation is an important antioxidant mechanism because transition metals such as iron can catalyze the formation of highly reactive hydroxyl radicals through Fenton-type reactions. By binding these metal ions, the extract can reduce the generation of

reactive oxygen species and thereby protect biological systems from oxidative damage. The observed ion chelating activity may be attributed to the presence of phenolic compounds, flavonoids, tannins, and other secondary metabolites capable of forming stable complexes with metal ions. The concentration-dependent increase in chelating activity indicates that *Digera muricata* possesses significant antioxidant potential through both free radical scavenging and metal ion chelation mechanisms (Shahidi, F., & Zhong, Y., 2015). In the hydroxyl radical scavenging assay, the extract showed 87.70% inhibition at the highest concentration tested, with an IC_{50} value of 56.0 µg/ml. Hydroxyl radicals are among the most reactive oxygen species and can cause extensive damage to DNA, proteins, and lipids. The substantial scavenging activity observed indicates that the hydro-ethanolic extract possesses compounds capable of protecting biological molecules from oxidative degradation. Similar antioxidant behaviour has been reported in phenolic-rich medicinal plant extracts (Halliwell, B et al., 1987). The hydrogen peroxide scavenging assay demonstrated 88.34% inhibition at 100 µg/ml with an IC_{50} value of 53.5 µg/ml. Hydrogen peroxide itself is relatively less reactive; however, it can generate highly toxic hydroxyl radicals in the presence of metal ions. Therefore, the ability of the extract to effectively scavenge hydrogen peroxide further confirms its antioxidant potential and protective role against oxidative stress (Prior R. L., et al., 2005). Hydrogen peroxide itself is not highly reactive; however, it can penetrate biological membranes and, in the presence of transition metal ions, generate highly reactive hydroxyl radicals through the Fenton reaction. Therefore, the ability of the extract to remove hydrogen peroxide may help prevent oxidative damage to cellular components such as proteins, lipids, and nucleic acids. The observed scavenging activity may be attributed to the presence of phenolic compounds, flavonoids, tannins, and other antioxidant phytochemicals present in *Digera muricata*. These compounds can donate electrons or hydrogen atoms to hydrogen peroxide, converting it into water and thereby reducing oxidative stress. The concentration-dependent increase in scavenging activity further confirms the role of these bioactive constituents in antioxidant defence mechanisms.

Overall, the hydro-ethanolic leaf extract exhibited potent antioxidant activity in all tested assays, with inhibition values exceeding 85% at 100 µg/ml. The dose-dependent increase in activity and relatively low IC_{50} values indicate strong free radical scavenging and metal-chelating properties. These findings support previous reports that *Digera muricata* leaves are a rich source of natural antioxidants and may be useful in preventing oxidative stress-related disorders through their

ability to neutralize reactive oxygen and nitrogen species.

CONCLUSION

The present study demonstrated that the hydro-ethanolic leaf extract of *Digera muricata* possesses significant *in vitro* antioxidant activity, as evidenced by its effective DPPH radical scavenging, nitric oxide scavenging, iron chelating, hydroxyl radical scavenging, and hydrogen peroxide scavenging activities. The extract exhibited a concentration-dependent increase in antioxidant activity across all assays, with high percentage inhibition values and relatively low IC₅₀ values, indicating strong free radical neutralizing potential. The observed antioxidant effects may be attributed to the presence of bioactive phytochemicals such as phenolics, flavonoids, tannins, and other secondary metabolites that act as electron donors, metal chelators, and reactive oxygen species scavengers. These compounds collectively contribute to the prevention of oxidative stress and cellular damage caused by free radicals. The findings are in agreement with previous studies that have reported the antioxidant potential of *Digera muricata* leaves and highlighted their rich phytochemical composition. Overall, the results suggest that *Digera muricata* leaf extract is a promising natural source of antioxidants and may have potential applications in the development of nutraceutical, pharmaceutical, and functional food products aimed at preventing oxidative stress-related disorders. Further studies involving phytochemical isolation, characterization of active compounds, and *in vivo* antioxidant investigations are recommended to validate its therapeutic potential and elucidate the underlying mechanisms of action.

ACKNOWLEDGEMENTS

The authors would like to thank Department of Biochemistry, VISTAS for providing the necessary facilities for our research work.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this article

CONFLICT OF INTEREST

There are no conflicts of interest.

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