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Free Radical Scavenging and Antioxidant Potential of *Rauvolfia serpentina* Root Extracts

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ABSTRACT
Background: Oxidative stress plays a major role in the pathogenesis of several chronic and degenerative diseases. Natural antioxidants derived from medicinal plants are increasingly preferred over synthetic antioxidants due to their safety and therapeutic potential. Rauvolfia serpentina is a well-known medicinal plant traditionally used for various health disorders, yet detailed antioxidant profiling of its root extracts remains limited.
Objective: The present study is aimed to evaluate the in vitro free radical scavenging and antioxidant potential of methanolic and ethyl acetate root crude extracts of R. serpentina.
Methods: The antioxidant activity was assessed using multiple in vitro assays, including DPPH, nitric oxide, hydroxyl radical, superoxide radical, hydrogen peroxide, ABTS radical scavenging assays, Total antioxidant capacity (Phosphomolybdenum method), and Reducing power assay. The extracts were tested at concentrations of 25–200 µg/mL, and ascorbic acid was used as the reference standard. Antioxidant activity was expressed as IC_{50} values and ascorbic acid equivalents.
Results: Both methanol and ethyl acetate extracts exhibited dose-dependent antioxidant activity in all assays. However, the methanolic root extract consistently showed significantly stronger free radical scavenging activity with lower IC_{50} values, higher total antioxidant capacity, and greater reducing power compared to the ethyl acetate extract. Ascorbic acid showed the highest activity among all tested samples.
Conclusion: The findings clearly indicate that the methanolic root crude extract of Rauvolfia serpentina possesses superior antioxidant potential, likely due to its higher content of polar bioactive phytochemicals. This study scientifically supports the use of R. serpentina roots as a promising natural source of antioxidants for managing oxidative stress-related disorders.

1. INTRODUCTION:

Oxidative stress is a major contributing factor in the initiation and progression of numerous chronic and degenerative diseases, including cancer, cardiovascular disorders, diabetes, neurodegenerative diseases, and aging-related complications. It results from an imbalance between the production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, and the body's antioxidant defense system (Halliwell & Gutteridge, 2007). Excess ROS can damage cellular lipids, proteins, and DNA, leading to altered cellular functions and disease development (Aruoma, 1998).

Antioxidants play a crucial role in neutralizing these free radicals by donating electrons or hydrogen atoms, thereby preventing oxidative damage.

Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in food and pharmaceutical industries, their long-term consumption has raised serious health concerns due to their potential toxic and carcinogenic effects (Ito *et al*., 1985). This has led to a growing global interest in identifying safer, natural antioxidants from plant sources.

Medicinal plants are rich reservoirs of natural antioxidants, primarily in the form of phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids. These secondary metabolites exhibit strong free radical scavenging, metal chelating, and reducing properties (Cai *et al*., 2004; Marinova *et al*., 2005). Among them, phenolics and flavonoids are especially important due to their ability to donate hydrogen atoms or electrons and stabilize free radicals (Harborne & Baxter, 1993; Okpuzar *et al*., 2009). Therefore, the evaluation of antioxidant potential of medicinal plant extracts has become a vital area of research in natural product drug discovery. *Rauvolfia serpentina* (L.) (*R. serpentina*) Benth. ex Kurz, commonly known as Indian snakeroot or Sarpagandha, is a highly valued medicinal plant belonging to the family Apocynaceae. It has a long history of use in Ayurvedic, Unani, and Siddha systems of medicine for the treatment of hypertension, insomnia, anxiety, epilepsy, fever, gastrointestinal disorders, and snakebite (Sen & Sharma, 2020). The plant is particularly well known for its indole alkaloid reserpine, which revolutionized the treatment of hypertension in modern medicine (Vakil, 1949). In addition to reserpine, other bioactive alkaloids such as ajmaline, serpentine, rescinnamine, and yohimbine have also been reported, contributing to its cardioprotective and neuropharmacological properties (Bhat *et al*., 2005).\

Apart from alkaloids, *R. serpentina* roots are also rich in phenolic compounds and flavonoids, which are known to possess strong antioxidant potential. Phenolic compounds exhibit multiple biological functions, including antioxidant, antimutagenic, and anticarcinogenic activities (Marinova *et al*.,2005).

Flavonoids, which constitute nearly half of all known phenolic compounds, have been reported to show a wide range of pharmacological activities such as antimicrobial, anti-inflammatory, anticancer, antiulcer, and cardioprotective effects (Dewick, 2001; Harborne & Baxter, 1993). These compounds exert their antioxidant properties primarily through free radical scavenging, inhibition of lipid peroxidation, and metal ion chelation (Okpuzar *et al*., 2009).

Previous studies have reported the antioxidant potential of *R. serpentina* root extracts using different *in vitro* models, particularly methanolic and ethanolic extracts, which showed significant free radical scavenging and reducing power activities (Bhat *et al*., 2005; Sen & Sharma, 2020). However, the antioxidant efficiency of plant extracts is strongly influenced by the type of solvent used for extraction, as solvent polarity affects the solubility of bioactive compounds, especially phenolics and flavonoids (Cai *et al*., 2004; Marinova *et al*., 2005). Therefore, comparative evaluation of selected solvent extracts is essential to identify the most potent antioxidant fraction.

In this context, the present study focuses on the free radical scavenging and antioxidant potential of selected *R. serpentina* root extracts, chosen based on their previously established bioactivity. The study aims to scientifically validate the antioxidant efficacy of these extracts using standard in vitro free radical scavenging assays, thereby strengthening the pharmacological significance of *R. serpentina* as a natural source of antioxidants and supporting its potential application in oxidative stress–related disorders.

1.0 MATERIAL AND METHODS:

1.1 Selection of Extracts and Preparation of Test Solutions

Based on previous antibacterial and phytochemical findings, the methanolic (ME) and ethyl acetate (EAE) root extracts of *R. serpentina* were selected for antioxidant evaluation. Each extract was dissolved in a minimal quantity of DMSO and further diluted with methanol to obtain working concentrations of 25, 50, 75, 100, 150, and 200 µg/mL. The final concentration of DMSO did not exceed 1% (v/v). Ascorbic acid was used as the standard antioxidant and prepared in the same concentration range.

1.2 Antioxidant activity

1.2.1 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Brand-Williams *et al*. (1995). A 0.1 mM DPPH solution was prepared in methanol. To 1.0 mL of DPPH solution, 1.0 mL of extract or ascorbic acid at different concentrations (25, 50, 75, 100, 150, and 200 µg/mL) was added. The control contained DPPH with methanol only. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH·scavenging·property·(%)·=·[OD$_0$–·OD$_1$/·OD$_0$]·X·100

OD0 is the optical density of the only reagent without plant extract, and OD1 is the optical density of the test sample. The results are shown as IC50.

1.2.2 Nitric Oxide (NO) Radical Scavenging Assay
The nitric oxide radical scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Green *et al*. (1982) and Marcocci *et al*. (1994). Briefly, 10 mM sodium nitroprusside in phosphate buffer saline (PBS, pH 7.4) was mixed with different concentrations of the extract and incubated at 25°C for 150 minutes. After incubation, 0.5 mL of the reaction mixture was mixed with 1.0 mL of sulfanilic acid reagent and incubated for 5 minutes, followed by the addition of 1.0 mL of naphthyl ethylenediamine dihydrochloride. The absorbance of the chromophore formed was measured at 546 nm against a reagent blank. Ascorbic acid was used as the reference standard.

Nitric oxide scavenging property (%) = [OD$_0$– OD$_1$/ OD$_0$] X 100

OD0 is the optical density of the only reagent without plant extract, and OD1 is the optical density of the test sample. The results are shown as IC50.

1.2.3 Hydroxyl Radical Scavenging Assay
The hydroxyl radical scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Halliwell *et al*. (1987) using the deoxyribose degradation assay. The reaction mixture contained $FeCl_3$ (100 µM), EDTA (100 µM), H_2O_2 (1 mM), deoxyribose (2.8 mM), phosphate buffer (20 mM, pH 7.4), and the extract at different concentrations. The mixture was incubated at 37°C for 1 hour. After incubation, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were added, and the mixture was heated at 100°C for 20 minutes. The absorbance of the resulting pink chromogen was measured at 532 nm. Ascorbic acid served as the positive control.

Hydroxyl Radical scavenging property (%) = [OD$_0$– OD$_1$/ OD$_0$] X 100

OD0 is the optical density of the only reagent without plant extract, and OD1 is the optical density of the test sample. The results are shown as IC50.

1.2.4 Superoxide Radical Scavenging Assay
The superoxide radical scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Nishikimi *et al*. (1972) using the PMS–NADH–NBT system. Superoxide radicals were generated in a reaction mixture

containing NADH, nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), and the extract at different concentrations. The reaction mixtures were incubated at room temperature, and the reduction of NBT was measured at 560 nm. Ascorbic acid was used as the reference standard.

Superoxide scavenging property (%) = [OD$_0$– OD$_1$/ OD$_0$] X 100

OD0 is the optical density of the only reagent without plant extract, and OD1 is the optical density of the test sample. The results are shown as IC50.

1.2.5 Hydrogen Peroxide Scavenging Assay
The hydrogen peroxide scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Ruch *et al*. (1989). A 40 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). To this solution, the extract at different concentrations was added and incubated for 10 minutes at room temperature. The absorbance was measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard.

Hydrogen peroxide scavenging property (%) = [OD$_0$– OD$_1$/ OD$_0$] X 100

OD0 is the optical density of the only reagent without plant extract, and OD1 is the optical density of the test sample. The results are shown as IC50.

1.2.6 ABTS Radical Cation Scavenging Assay
The ABTS radical cation scavenging activity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Re *et al*. (1999). The $ABTS^{\bullet+}$ radical was generated by reacting ABTS stock solution with potassium persulfate and incubating the mixture in the dark for 12–16 hours.

Before use, the $ABTS^+$ working solution was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After adding the extract at different concentrations, the absorbance was measured at 734 nm. Ascorbic acid was used as the standard antioxidant.

1.2.7 Total Antioxidant Capacity (Phosphomolybdenum Method)
The total antioxidant capacity of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Prieto *et al*.

(1999). 0.3 mL of extract was mixed with 3.0 mL of phosphomolybdenum reagent (containing sulfuric acid, sodium phosphate, and ammonium molybdate). The reaction mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

1.2.8 Reducing Power Assay

The reducing power of the methanolic and ethyl acetate root crude extracts of *R. serpentina* was determined by the method described by Oyaizu (1986). The extract was mixed with phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 minutes. After incubation, trichloroacetic acid (TCA) was added and the reaction mixture was centrifuged. The upper layer was mixed with ferric chloride ($FeCl_3$), and the absorbance was measured at 700 nm. Increased absorbance indicated higher reducing power.

1.3 Statistical Analysis

All experiments were conducted in triplicate (n = 3). Results were expressed as mean ± SD**.** IC_{50} values were calculated from dose–response curves. Significance was evaluated using one- way ANOVA at $p < 0.05$.

2.0 Results

The *in vitro* antioxidant activity of the methanolic and ethyl acetate root crude extracts of *Rauvolfia serpentina* was evaluated using eight different free radical scavenging and reducing power assays. The results were expressed in terms of IC_{50} values for radical scavenging assays and as absorbance or ascorbic acid equivalents for total antioxidant capacity and reducing power. A lower IC_{50} value indicates stronger antioxidant potential. The results demonstrated a clear dose-dependent increase in free radical scavenging activity for both extracts, with the methanolic extract consistently exhibiting superior antioxidant activity compared to the ethyl acetate extract in all assays (**Table 1**).

2.1 DPPH Radical Scavenging Activity

The methanolic root extract of *R. serpentina* showed strong DPPH radical scavenging activity with an IC_{50} value of 48.2 ± 1.4 µg/mL, whereas the ethyl acetate extract exhibited a comparatively weaker activity with an IC_{50} value of 72.6 ± 2.1 µg/mL. The reference standard, ascorbic acid, exhibited a much lower IC_{50} value (21.4 ± 0.9 µg/mL), confirming its high free radical scavenging efficiency. The results clearly indicate that the methanolic extract possesses significantly greater DPPH scavenging potential than the ethyl acetate extract.

2.2 Nitric Oxide Radical Scavenging Activity

In the nitric oxide scavenging assay, the methanolic extract demonstrated higher inhibitory activity with an IC_{50} value of 54.7 ± 1.9 µg/mL, while the ethyl acetate extract showed weaker activity with an IC_{50} value of 80.3 ± 2.4 µg/mL. Ascorbic acid showed strong nitric oxide scavenging ability with an IC_{50} value of 24.6 ± 1.1 µg/mL. These findings further support the superior antioxidant efficiency of the methanolic extract.

2.3 Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are among the most reactive oxygen species capable of causing severe oxidative damage. The methanolic extract exhibited strong hydroxyl radical scavenging activity with an IC_{50} value of 45.9 ± 1.6 µg/mL, which was significantly lower than that of the ethyl acetate extract (69.5 ± 2.0 µg/mL). Ascorbic acid exhibited the highest scavenging efficiency with the lowest IC_{50} value (19.8 ± 0.7 µg/mL). The lower IC_{50} value of the methanolic extract indicates its greater ability to neutralize hydroxyl radicals.

2.4 Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the methanolic extract was found to be stronger (IC_{50} = 52.1 ± 1.8 µg/mL) compared to the ethyl acetate extract (IC_{50} = 77.4 ± 2.2 µg/mL). Ascorbic acid showed an IC_{50} value of 23.9 ± 0.8 µg/mL. The results demonstrate that the methanolic extract is more effective in scavenging superoxide anions than the ethyl acetate extract.

2.5 Hydrogen Peroxide Scavenging Activity

The methanolic extract also exhibited higher hydrogen peroxide scavenging activity with an IC_{50} value of 61.3 ± 2.0 µg/mL, whereas the ethyl acetate extract showed relatively lower scavenging ability (89.6 ± 2.8 µg/mL). Ascorbic acid displayed strong hydrogen peroxide scavenging activity with an IC_{50} value of 26.1 ± 1.0 µg/mL. These results indicate that the methanolic extract is more efficient in preventing hydrogen peroxide-induced oxidative damage.

2.6 ABTS Radical Cation Scavenging Activity

In the ABTS radical scavenging assay, the methanolic extract showed a significantly lower IC_{50} value (43.6 ± 1.3 µg/mL) compared to the ethyl acetate extract (66.2 ± 1.9 µg/mL), indicating higher scavenging potential. Ascorbic acid exhibited the strongest activity with an IC_{50} value of 18.5 ± 0.6 µg/mL. The superior ABTS scavenging ability of the methanolic extract further confirms its strong antioxidant potential.

2.7 Total Antioxidant Capacity

The total antioxidant capacity of the extracts, determined by the Phosphomolybdenum method,

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revealed that the methanolic extract possessed significantly higher antioxidant capacity (96.4 ± 2.5 mg AAE/g extract) compared to the ethyl acetate extract (71.8 ± 2.1 mg AAE/g extract). The standard ascorbic acid showed the highest value (112.3 ± 3.2 mg AAE/g). These findings indicate the higher overall antioxidant strength of the methanolic extract.

2.8 Reducing Power Assay

The reducing power of the extracts increased with increasing concentration. At 200 µg/mL, the methanolic extract showed significantly higher absorbance (1.86 ± 0.05) compared to the ethyl acetate extract (1.24 ± 0.04), indicating greater electron-donating capacity and stronger reducing ability. Ascorbic acid showed the highest reducing power (2.12 ± 0.06). The higher reducing power of the methanolic extract further supports its stronger antioxidant nature.

Table 1.0 *In Vitro* Antioxidant Activity of Methanolic and Ethyl Acetate Root Crude Extracts of *R. serpentina*

Antioxidant Assay	ME	EAE	Ascorbic Acid
DPPH Radical	48.2 ± 1.4	72.6 ± 2.1	21.4 ± 0.9
Nitric Oxide Radical	54.7 ± 1.9	80.3 ± 2.4	24.6 ± 1.1
Hydroxyl Radical	45.9 ± 1.6	69.5 ± 2.0	19.8 ± 0.7
Superoxide Radical	52.1 ± 1.8	77.4 ± 2.2	23.9 ± 0.8
Hydrogen Peroxide	61.3 ± 2.0	89.6 ± 2.8	26.1 ± 1.0
ABTS Radical	43.6 ± 1.3	66.2 ± 1.9	18.5 ± 0.6
Total Antioxidant Capacity*	96.4 ± 2.5	71.8 ± 2.1	112.3 ± 3.2
Reducing Power	1.86 ± 0.05	1.24 ± 0.04	2.12 ± 0.06

Values are expressed as mean ± SD (n = 3). IC_{50} **(µg/mL)** represents the concentration required to inhibit 50% of free radicals. *Total antioxidant capacity is expressed as mg ascorbic acid equivalent (AAE)/g extract.

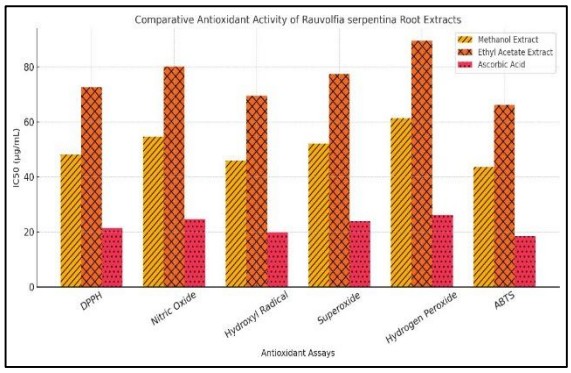

Fig 1.0. Comparative IC_{50} values of methanolic and ethyl acetate root crude extracts of *R. serpentina* against different free radicals using various *in vitro* antioxidant assays.

3.0 DISCUSSION:

In the present study, the *in vitro* antioxidant activity of methanolic and ethyl acetate root crude extracts of *R. serpentina* was systematically evaluated using multiple free radical scavenging and reducing power assays. Across all tested systems—DPPH, nitric oxide, hydroxyl radical, superoxide anion, hydrogen peroxide and ABTS radical cation,the methanolic root extract consistently exhibited lower IC_{50} values than the ethyl acetate extract, indicating a higher free radical scavenging efficiency. In DPPH assay, the methanolic extract demonstrates a markedly lower IC_{50} compared to the ethyl acetate extract, while ascorbic acid showed the strongest activity as expected. A similar trend was observed in Nitric oxide, Hydroxyl, superoxide and Hydrogen peroxide scavenging assays, where methanol extract always requires a lower concentration to achieve 50% inhibition than the ethyl acetate fraction. This pattern clearly suggests that the methanolic extract of *R. serpentina* roots is richer in effective antioxidant constituents capable of neutralizing a broad spectrum of reactive oxygen species (ROS).

The ABTS assay, which can assess both hydrophilic and lipophilic antioxidants, also confirms the superior scavenging capacity of the methanolic extract. This is further supported by the higher total antioxidant capacity (Phosphomolybdenum method) and greater Reducing power (A_{700}) observed for the methanolic fraction compared to the ethyl acetate extract. Since both total antioxidant capacity and reducing power are closely linked to a sample's electron- donating and redox properties, these results indicate that the methanolic extract is more efficient in acting as a primary antioxidant and reducing agent.

The present findings agree well with earlier reports that solvent polarity critically influences the antioxidant potency of plant extracts, as the polar solvents such as methanol and ethanol extract phenolic compounds and flavonoids more efficiently and are recognized as major contributors to antioxidant activity (Zheng & Wang, 2001; Cai *et al*., 2004;Li *et al*., 2008). Phenolic compounds act as chain-breaking antioxidants by donating hydrogen atoms or electrons to free radicals and stabilizing them, while flavonoids can additionally chelate transition metals and inhibit lipid peroxidation (Halliwell & Gutteridge, 2007; Li *et al*., 2017). Several authors have reported significant antioxidant activity of *R. serpentina* extracts, particularly in methanolic and aqueous methanolic fractions. A comparative study on *Rauvolfia* species

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showed that *R. serpentina* exhibited the highest DPPH radical scavenging activity and elevated pigment and vitamin E content among the tested species, emphasizing its strong antioxidant potential (Nair *et al*., 2012). Methanolic extract of *R. serpentina* leaves and rhizomes has also been reported to display notable DPPH, superoxide scavenging and reducing power activities, often associated with high levels of carotenoids, tocopherols, flavonoids and phenolics (Gupta & Gupta, 2015; Kishore *et al*., 2018). Recent work on methanolic leaf extract has further confirmed that *R. serpentina* possesses substantial antioxidant capacity, which correlates with its phytochemical complexity (Alshahrani *et al*., 2021; Roy *et al*., 2023).

Our observation that the methanolic root extract shows stronger DPPH and ABTS scavenging than the ethyl acetate extract is also consistent with broader plant antioxidant literature, where DPPH and ABTS methods are widely used and highly sensitive to phenolic content (Re *et al*., 1999; Gülçin, 2010; Gülçin, 2023). Studies screening large panels of medicinal plants and herbs have repeatedly demonstrated strong correlations between total phenolic content and free radical scavenging capacity measured by DPPH, ABTS and FRAP assays (Zheng & Wang, 2001; Cai *et al*., 2004; Li et *al*., 2008; Piluzza & Bullitta, 2011).

Furthermore, the multi-assay approach in our study covering DPPH, ABTS, nitric oxide, hydroxyl, superoxide, and hydrogen peroxide radicals, as well as total antioxidant capacity and reducing power, is in line with recommendations that no single assay fully describes antioxidant behavior, and that different ROS/reactive species must be assessed separately (Loganayaki *et al*., 2011; Li *et al*., 2017; Seon *et al*., 2021). The observation that the methanolic extract performs consistently better than the ethyl acetate extract across this diverse panel of assays strongly suggests that it contains a broader and more potent spectrum of antioxidant molecules**.** In addition, previous pharmacological studies on *R. serpentina* have shown that methanolic root extracts can ameliorate oxidative stress in vivo by modulating enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, while also reducing lipid peroxidation in animal models (Azmi *et al*., 2015; Biswar *et al*., 2021). These in vivo findings, together with the present in vitro results, reinforce the view that *R. serpentina* is a biologically relevant source of antioxidant principles**.**

It is also noteworthy that related antioxidant activity has been reported from *R. serpentina*- associated systems such as endophytic bacteria isolated from its leaves, which exhibit antibacterial and antioxidant potentials, indicating that the plant microbe holobiont may co- contribute to the observed bioactivities (Lata *et al*., 2025).

4.0 CONCLUSION:

The present study clearly demonstrates that the methanolic root crude extract of *R. serpentina* possesses significantly higher free radical scavenging and antioxidant potential than the ethyl acetate extract across multiple *in vitro* antioxidant assays. The superior activity of the methanolic extract may be attributed to its higher ability to extract polar antioxidant phytochemicals responsible for neutralizing reactive oxygen species. These findings scientifically validate the antioxidant potential of *R. serpentina* roots and support their possible application as a natural source of antioxidants for managing oxidative stress-related disorders. Further studies involving isolation of active compounds and *in vivo* validation are warranted to confirm their therapeutic potential.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this research work.

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