Journal of Animal Environment

(E-ISSN 2717-1396, ISSN 2717-1388)

Original Research Paper

Exploring the Therapeutic Potential of *Rauwolfia Serpentina*: In Vitro Antioxidant and Anti-inflammatory Activity Evaluation

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Abstract:

Background: Oxidative stress and chronic inflammation are major contributors to the development of several pathological conditions, including cardiovascular, neurodegenerative, and inflammatory disorders. Natural medicinal plants rich in bioactive phytochemicals have gained attention as potential therapeutic agents. *Rauwolfia serpentina* (L.) Benth. ex Kurz is a traditional medicinal plant known for its diverse pharmacological properties, yet its antioxidant and anti-inflammatory potential require further scientific validation.

Objective: The present study aimed to evaluate the phytochemical profile, antioxidant activity, cytotoxicity, and in vitro anti-inflammatory potential of methanolic root extract of *Rauwolfia serpentina*.

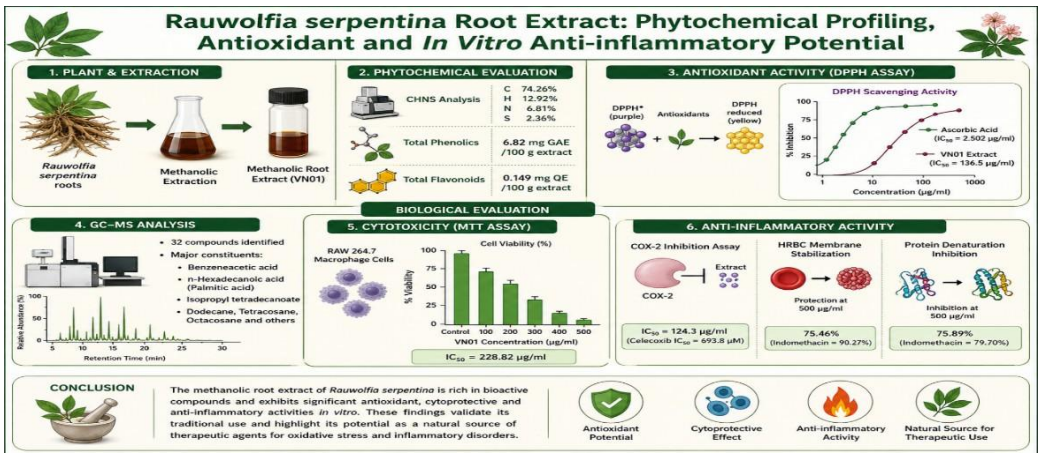
Materials and Methods: Roots of *Rauwolfia serpentina* were extracted using methanol and subjected to phytochemical, CHNS, total phenolic, and total flavonoid analyses. Antioxidant activity was assessed using the DPPH free radical scavenging assay. GC–MS analysis was performed to identify bioactive constituents. Cytotoxicity was evaluated using the MTT assay on RAW 264.7 macrophage cells. Anti-inflammatory activity was determined through cyclooxygenase-2 (COX-2) inhibition, human red blood cell (HRBC) membrane stabilization, and protein denaturation inhibition assays.

Results: The methanolic extract exhibited appreciable phenolic and flavonoid contents and demonstrated moderate antioxidant activity with a DPPH IC_{50} value of 136.5 µg/mL. GC–MS analysis identified 32 compounds, including phenylacetic acid, palmitic acid, and isopropyl myristate. The extract showed concentration-dependent cytotoxicity against RAW 264.7 cells with an IC_{50} value of 228.82 µg/mL. Significant anti-inflammatory activity was observed, with COX-2 inhibitory activity (IC_{50} 124.3 µg/mL), HRBC membrane stabilization (75.46%), and protein denaturation inhibition (75.89%) at 500 µg/mL.

Conclusion: The methanolic root extract of *Rauwolfia serpentina* possesses notable antioxidant and anti-inflammatory activities, which may be attributed to its rich phytochemical composition. These findings support its traditional medicinal use and suggest its potential as a natural source for developing therapeutic agents against oxidative stress and inflammatory disorders.

Keywords- *Rauwolfia serpentina,* Phytochemical profile, Antioxidant activity, Cytotoxicity, Anti-inflammatory potential, Methanolic root extract.

Graphical Abstract



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Received: 25th May, 2026; Revised: 6th June, 2026; Accepted: 8th June, 2026; Available Online: 29rd July, 2026

(DOI): 10.70102/AEJ.2026.18.2s.23

Introduction

Inflammation is the normal response of body's immune system that occurs in vascular tissues against harmful stimuli such as pathogens, cellular damage and irritants [1]. Chronic inflammation is usually associated with an increase in reactive nitrogen and oxygen species production bringing about an oxidative stress initiated by an imbalance between reactive oxygen species and the biological system's defence able to eliminate these free radicals [2]. This oxidative stress has been involved in several diseases including cancer, neurodegenerative diseases, atherosclerosis, malaria, chronic fatigue syndrome and rheumatoid arthritis [3]. Recently there has been an extensive interest in the therapeutic potential of medicinal plants as antioxidants in scavenging such free radical-induced tissue injury [4]. Rauwolfia serpentina is a traditional medicine used by Indians for a long time. Because of its serpentine shape, the drug is known as Sarpgandha. Despite having more than 50 alkaloids, the primary alkaloid in Rauwolfia serpentina is reserpine. Reserpine, even at lower doses, is an effective antihypertensive. Rauwolfia serpentina has a wide variety of medicinal uses, including lowering blood pressure and protecting the liver, but it also has sedative, antipsychotic, antidiarrheal, and anticancer (in breast) effects, among many others. The herb Rauwolfia serpentina contains all four of the main indole alkaloids, but we're mainly interested in the root-concentrated active alkaloid reserpine here because of its role in the plant's antihypertensive effects. In order for reserpine to have an antihypertensive effect, it needs to be administered at a much lower level. At higher doses, it can cause serious adverse effects such as drowsiness, depression, anxiety, nausea, bradycardia, bronchospasm, and withdrawal psychosis [5].

Methodology

Plant material collection and identification

Rauwolfia Serpentina roots were employed in this investigation. The authors gathered the samples at Maharana Pratap College of Pharmacy's and approved the plant Central Institute of Medicinal & Aromatic Plants in Lucknow. They placed a voucher specimen in the Plant Systematics Laboratory **(CIMAP-3685).**

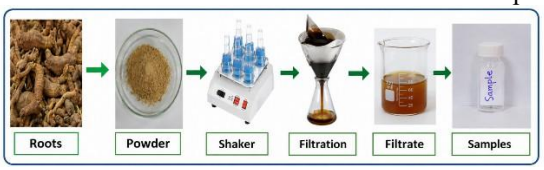
Extraction

5 Gm of sample was taken and mixed with 50 ml of solvent (Absolute Methanol). The sample mixture was then incubated on a rocker shaker for 24 hours. Then the extract was filtered through Whatman filter paper 1, and the extract was then completely dried in the oven at 40°C. The Extract were then collected in micro centrifuge tube and stored at 4°C [6].

Yield **%=Weight of dry extract/ Weight of dry roots powder ×100**

Chemicals: All the chemical were of AR grade and were purchased from Vikas Sales Corporation, India.

CHNS analysis: On Unicube (Germany, Elementar), an elemental analysis of *Rauwolfia Serpentina* roots powder was done. CHNS was quantified and analysed at the Energetic Materials Lab of IIT. Kanpur



% RSA= (ABS control-Abs Sample)/Abs control) x100

Figure-1 Methanolic extractions of *Rauwolfia Serpentina* roots

Qualitative phytochemical screening: To confirm the existence of different bioactive components, a standard qualitative investigation of the methanolic extractions extract was performed.

Determination of total flavonoid contents: Aluminum chloride colorimetric techniques were used to determine flavonoid concentrations. *Rauwolfia Serpentina* roots extract was sonicated for 15 minutes at maximum power in a volume of 10 milliliters of 80% methanol before being filtered using Whatmann filter paper No.42 (125 millimeters). Then, in a test tube, they combined 0.3 mL of filtrate, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M NaNO2 and 0.15 mL of 0.3 M AlCl3-6H2O. The absorbance was taken at 510 nm after adding 1 mL of 1M NaOH and letting it sit for 5 minutes. Using quercetin quantities that were previously determined, a standard calibration curve was generated. Absorbance was used to measure total flavonoid concentration which was then expressed as mg quercetin equivalent per gram of extract (mg QE/g powder) [7-8].

Determination of total phenolic compound: The extracts' total soluble phenolic content was calculated using a modified version of the Folin-Ciocalteu technique15. The sample solution (1.0 mg/mL) and the 10% Folin-Ciocalteu solution (400 µll) were combined in a volumetric flask and incubated for 10 minutes at room temperature before being combined with the 7% Na2CO3 solution (0.2 mL). Finally, 10 mL of the solution was prepared by diluting the original solution with deionized distilled water in a volumetric flask. After 2 hours at room temperature, the absorbance of the combination was

measured at 725 nm to determine its stability. Using the equation y = 0.2177x+0.0005, r2=0.999, we were able to convert the total phenolic components in the extract into grams of gallic acid equivalent (GAE). If x represented concentration and y represented absorbance, then gallic acid equivalents per gram of extract (mg GAE/g extract) were used to determine total phenolics [9-10].

IN VITRO ANTIOXIDANT ACTIVITY

DPPH Scavenging Assay

To evaluate antioxidant activity, 10 µL of different concentrations of test samples or standard (Ascorbic Acid, SRL Cat. No. 23006) were mixed with 200 µL of 0.1 mM DPPH solution (SRL Chem, Cat. No. SR-29128) in a 96-well plate. Reactions were prepared in quadruplicate; duplicate blanks (sample + methanol without DPPH), red blanks (without DPPH), and untreated wells served as controls. Plates were incubated in the dark for 30 min, and absorbance was read at 517 nm using a microplate reader (iMark, Bio-Rad). A combination of test solution with 20 µL deionized water served as control. Scavenging activity (%) was calculated relative to control. IC_{50} values were derived using GraphPad Prism 6 by plotting inhibition (%) vs. sample concentration [11].

GC-MS Analysis

GC-MS analysis of methanolic extracts was performed using a PerkinElmer Clarus 600 system equipped with an Rtx-5MS capillary column. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection volume: 1 µL, split ratio: 10:1, ionization energy: 70 eV, scan range: 40–650 m/z, scan time: 0.2 sec. Injector temperature: 260°C. Oven temp held at 50°C for 3 min, then ramped to 300°C at 10°C/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST and Wiley-8 libraries [12-13].

CELL VIABILITY ASSAY

MTT assay

Cytotoxicity of the provided samples on Raw 264.7 cell line (Passage No. 23, Procured from NCCS Pune, India, RRID- CVCL_0019) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO2. Next day cells were treated from different concentrations of the samples. Stock solution of samples was prepared in DMSO and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (Air- jacketed CO2 incubator-Heal force-HF90) for 2 h. Cells without treatment were considered as Control and cells without MTT were considered as Blank. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO –SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm. IC_{50} was calculated by using Graph Pad Prism -6. [14].

Calculation

% Viable cells = (Atest / AControl) *100

(Atest = Absorbance of test sample)

IN VITRO MODELS OF ANTI-INFLAMMATORY ACTIVITY

Enzyme Inhibition Assay - Cox-II

Sample dilution was prepared. Buffer (Tris Cl buffer, 100mM, pH 8.0 (SRL Chem-Cat no:71033) were prepared. Reaction buffer (Enzyme in Tris/heme/phenol; 100mM/1µM/1µM buffer-Bovine Hemin Chloride (SRL- Cat no.-78372) were placed in defined well of a 96-well plate. The reaction was initiated by adding 5 µl substrate was added to each reaction mixture (Arachidonic acid, 10 mM- SRL Cat no.- 20975) and 5 µl TMPD solution (17mM- HiMedia GRM445-5G) and then plate was incubated (Basil Scientific Corp. India-Incubator) at room temperature for 10 minutes and absorbance was taken at 595 nm using ELISA micro plate reader (iMark, BioRad). Inhibitor, Celecoxib (2500 µM final Concentration- TCI-C2816) was used as a positive control. IC-50 was calculated using Software Graph Pad Prism 6 [15].

Calculations

% Inhibition = ($A_{Control}$ - A_{test} /$A_{Control}$) ×100

$A_{Control}$ = Absorbance of control A_{test} = Absorbance of sample

Inhibition of albumin denaturation

The Inhibition of albumin denaturation was assessed according to the modified method of (Chandra et al., 2012). Briefly, one mL of 1% bovine serum albumin prepared in PBS (phosphate-buffered saline, pH 6.4) was added to one mL of varying concentrations of plant filtrate. This mixture was left at ambient temperature for 20 min and then heated at 70°C for 5 min. The resulting mixture was cooled down to the ambient temperature and their turbidities were read at 660 nm. The same procedure was repeated using doubledistilled water and the Diclofenac as control and standard respectively. The Percentage inhibition (IP%) of protein denaturation was calculated as follows [16].

Calculations

% Inhibition = (A Control - A test /A Control) ×100

A Control = Absorbance of control A test = Absorbance of sample

Human red blood cell (HRBC) membrane stabilization assay:

The in vitro anti-inflammatory activity of *Rauwolfia Serpentina* roots was determined by the Human Red Blood Cell (HRBC) membrane stabilization method. The blood sample was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks before the experiment, added with an equal amount of Alsever's solution. This mixture was washed thrice with sterile saline till the supernatant was clear and colorless. The packed cellular content was formulated as 10 % v/v suspension with sterile isosaline. 1 ml of different concentrations (100-500 µg/ml) of *Rauwolfia Serpentina* roots extract and standard indomethacin was mixed with 1 ml of phosphate buffer, 0.5 ml of HRBC suspension, and 2 ml of hyposaline. After 30 min incubation at 370C, the reaction mixture was centrifuged at 3000 rpm for 10 min. The supernatant absorbance was observed spectrophotometrically at 560 nm. The percentage of hemolysis was estimated by considering the percentage of hemolysis of control as 100%. The percentage of protection/ percentage inhibition of hemolysis were evaluated using the formula [17].

$$\%Protection = 100 - \frac{Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Statistical analysis: The statistical analysis was performed in IBM SPSS version 18 (SPSS version 18.0; IBM Corporation, Armonk, NY, USA). The entire assay was performed in triplicate and the values were expressed as mean ± standard deviation (SD). Descriptive statistics were used for continuous variables and expressed in mean and standard deviation. A test of normality was applied.

Result and Discussion

The Hexane leaver extracts of PS-01had yields in percentages of 3.34% respectively in table-1.

Table-1 Represents recovery rate of methanolic roots extracts

S. No.	Sample Code	Weight of Powder (mg)	Weight of Extract(mg)	% Recovery
1	VN-01 *Rauwolfia Serpentina*	5000	29	0.58

CHNS analysis: A compound's elemental makeup may be determined using elemental analysis. Carbon, hydrogen, nitrogen and sulphur were identified in the study. Both nitrogen and sulphur are essential macronutrients for plant development. Plants benefit from the presence of certain elements, while humans benefit from obtaining those elements via ingestion. Carbohydrates and hydrocarbons may be extracted from plants with a high carbon and hydrogen content. Because of its high nitrogen and sulphur content, this plant is likely a good source of proteins and vitamins, both of which are crucial to good health. Table 2 demonstrates that the required percentage of components may be found in *Rauwolfia Serpentina* root powder.

Table-2 Elements Percentage composition of *Rauwolfia Serpentina* roots powder

S.N.	Elements	Results %
1.	Carbon	74.26%
2.	Hydrogen	12.923 %
3.	Nitrogen	6.81%
4.	Sulphur	2.359%

Determination of total phenolic and flavonoid compounds: Table 3 displays the phenolic and flavonoid content of *Rauwolfia Serpentina* roots powder. There were more total phenolics in the methanolic extract.

Table-3 Phenolic and flavonoid contents of *Rauwolfia Serpentina* roots powder

Herbal extracts	Total phenolic contents (mg gallic acid/100g extract)	Total flavonoids (mg Quercetin/100g extract)
Hexane extract	6.2±0.38*	0149±0.02*

Oxidative stress has been linked to age-related neurodegenerative diseases in a number of studies. Numerous studies have also examined the protective effects of antioxidants in preventing or reducing neuronal death that occurs in the pathophysiology of this disorder. Indicators of a compound's antioxidant potential also include its total antioxidant activity and ability to scavenge radicals. To determine the plant extracts' ability to act as antioxidants, activities—DPPH evaluated. The 1, 2-diphenyl-2-picryl hydroxyl radical (DPPH) was used to examine the antioxidant activity of many extracts. Table 4presents the results.

Table-4DPPH Scavenging Assay

Sample code	IC_{50} value(µg/ml) (Mean ± SEM)
Ascorbic Acid	2.502±0.02
VN01	136.5±0.05

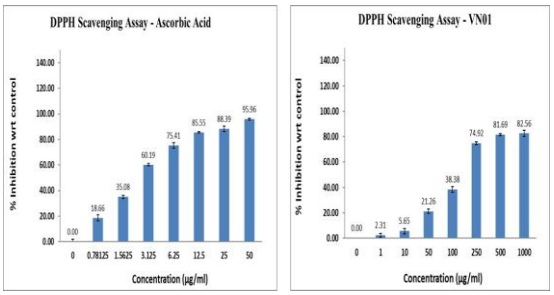


Figure-2 Graph represents the DPPH Scavenging Assay of *Rauwolfia Serpentina* roots powder Extract

Based on the results obtained from the experimental work, Antioxidant activity (DPPH Assay) was estimated in sample, and 50% inhibitory concentration (*IC50*) was mentioned in table 1. Sample – VN01 was found to be moderately active. 136.5 µg of sample-VN01 was found equivalent to 2.502 µg of standard Ascorbic acid. "The standard compound, being a purified compound and highly active substance, was assessed using a lower concentration range (0 to 50 µg/ml). In contrast, the sample's potency remains unbound; therefore, a broader concentration range (0 to 1000 µg/ml) was employed for the sample analysis.

Identification of components*:*

Identification was done using the calculated fragments, molecular mass, and molecular structure. The NIST database and the Willy8-library, which has more than 62,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy8 collection and the NIST library. This is done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps determine the most effective methods for removing harmful compounds. The test materials' constituent parts were determined (Table 5, and Figure 3).

Table 5- Compounds of *Rauwolfia Serpentina* roots powder Extract

Peak	R. Time	Area	Area%	Name
1	10.439	241802	1.15	**Dodecane**
2	11.030	39487	0.19	**Propyl methyl diketone**
3	11.346	163007	0.78	**Decane**
4	11.533	345701	1.65	**Hexadecane**
5	12.867	16221	0.08	**Di-tert-butylethane**
6	13.266	447166	2.13	**Tetradecane**
7	13.468	81707	0.39	**Tridecane**
8	13.910	37304	0.18	**Undecane**
9	14.049	381951	1.82	**4-Propionylbrenzcatechin**
10	14.406	457972	2.18	**Heptadecane**
11	14.685	82410	0.39	**Phenol, 3,5-bis(1,1-dimethylethyl)-**
12	16.084	89618	0.43	**n-Pentadecane ($C_{15}H_{32}$)**
13	16.366	73808	0.35	**1,1-Hexylenedioxybutane**
14	16.869	117358	0.56	**3,7-Dimethylundecane ($C_{13}H_{28}$)**
15	18.273	3673930	17.52	**isopropyl tetradecanoate**
16	18.413	87189	0.42	**Methyl 2-oxooctadecanoate (C19H36O3)**
17	18.529	144788	0.69	**1,3-Propanediol, 2-dodecyl**
18	19.017	23975	0.11	**2-Ethylbutyl hexanoate**
19	19.114	83135	0.40	**Octacosane ($C_{28}H_{58}$)**
20	19.201	220504	1.05	**nonadecane**
21	19.396	7617666	36.32	**Benzeneacetic acid,**
22	19.793	1950982	9.30	**n-Hexadecanoic acid**
23	21.087	126225	0.60	**Dodecanenitrile / Lauronitrile ($C_{12}H_{23}N$)**
24	22.688	84338	0.40	**1-tridecanol**
25	23.070	154649	0.74	**Tetracosane ($C_{24}H_{50}$)**
26	23.514	56380	0.27	**butyl stearate**
27	24.228	63572	0.30	**Ethyl 2-acetyltetradecanoate**
28	24.375	94476	0.45	**Ethyl 3-hydroxyoctadecanoate**
29	24.562	114721	0.55	**Glycerol 1-palmitate**
30	24.757	156765	0.75	**tetracosane**
31	25.207	51291	0.24	**1-Iodo-2-methylnonane**
32	**26.133**	**33293**	**0.16**	**1-Fluorononane**

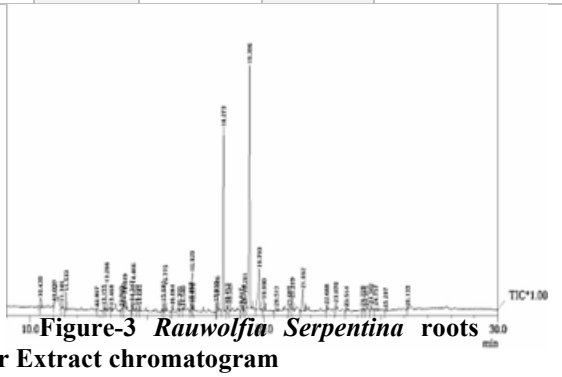


Figure-3 *Rauwolfia Serpentina* roots powder Extract chromatogram

The GC–MS analysis of sample VN01 revealed the presence of 32 compounds. Out of these, 29 plant compounds were identified, which are mentioned in the above table.

Cell Viability and Morphological observation

The cell viability assay was performed on a triplenegative Raw 264.7 cell line taken as the control group. In the present study, different concentrations (100µg - 500 µg) of standard drug and plant extract were taken to study morphological changes and cell growth inhibition in a cell line. An increase in the cell viability was observed at minimum concentration only. As the concentration increases, the viability of cells was decreased. Among three tested extracts, the methanol extract of *Rauwolfia Serpentina* exhibited prominent activity with a significant IC_{50} value of 228.82µg.

Table.6. Comparison of Effect of methanol solvent extracts of experimental plants on Raw 264.7 cell line Cell viability

Treatment	Cell Line	Concent ration in µg	Cell Viability in Percentage (%)	IC50 in µg
Methanol extract of *Rauwolfia Serpentina*	Raw 264.7	100	68.7142±0.015	228.82
		200	56.0645±0.0165	
		300	32.4310±0.0215	
		400	13.4825±0.0185	
		500	6.9234±0.0015	
		200	67.0900±0.0105	
		300	34.0551±0.0420	
		400	17.7782±0.0125	
		500	5.4138±0.0090	
Standard Drug Penicillin	Raw 264.7	20	6.9755±0.0050	≥15

I

Based on the results obtained from the study, Enzyme Inhibition Activity (COX-II) was estimated in samples and 50% Inhibitory concentration as mentioned in table 1. Sample- VN-01 was found to be moderately active as compared to standard – Celecoxib. 124.3µg of the sample- VN-01 was found to be equivalent to 693.8µM of standard- Celecoxib. Higher is the IC_{50}, lower be inhibition activity in table-7.

Table 7: Represents the Enzyme Inhibition Activity

Sample Code	IC_{50} value (Mean ± SEM)
Celecoxib	693.8±0.02 (µM)
VN-01	124.3±0.02 (µg/ml)

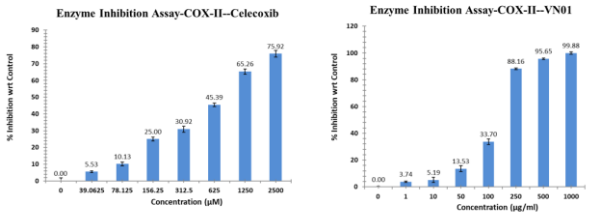


Figure-4 Represents the Enzyme Inhibition Activity of *Rauwolfia Serpentina* roots powder

The treatment of *Rauwolfia Serpentina roots powder m*ethanolic extract showed dose-dependent membrane stabilization action the maximum activity (75.46 ± 2.58) was observed at the concentration 500 µg/ ml. The standard diclofenac showed maximum membrane protection (90.27 ± 1.08) activity at the concentration 500 µg/ml, comparably the treatment of G *Rauwolfia Serpentina roots powder m*ethanolic extract showed less percentage inhibition of hemolysis compared to the standard indomethacin. The treatment of *Rauwolfia Serpentina roots powder m*ethanolic extract exhibited remarkable anti-inflammatory activity by stabilizing the RBC membrane, preventing the discharge of lytic enzymes and other inflammation mediators (Table 8). The results in table 7 shows that maximum (75.89± 7.89) percentage denaturation inhibition was observed in the *Rauwolfia Serpentina roots powder m*ethanolic extract 500 µg/ml treatment, when compared to standard indomethacin it shows maximum inhibition (79.70± 2.81) at the concentration of 500 µg/ml which is almost equal. The *Rauwolfia Serpentina roots powder m*ethanolic extract showed dose dependent protein denaturation inhibition property. As results the protein denaturation inhibition capacity confirms the anti-inflammatory property of *Rauwolfia Serpentina roots powder m*ethanolic extract.

Table 8. Human red blood cell membrane stabilization assay and Protein denaturation inhibition assay of *Rauwolfia Serpentina roots powder m*ethanolic extract

S. No.	Concentration (µg/ml)	Human red blood cell membrane stabilization assay		Protein denaturation inhibition assay	
		% Protection		% Protection	
		*Rauwolfia Serpentina roots powder m*ethanolic extract	Indomethacin	*Rauwolfia Serpentina roots powder m*ethanolic extract	Indomethacin
1.	100	23.51 ± 1.01	48.11 ± 2.77	18.88 ± 1.42	24.71 ± 3.31
2.	200	34.25 ± 0.98	66.96 ± 2.54	27.12 ± 1.32	39.14 ± 6.04
3.	300	40.19 ± 1.50	75.39 ± 7.80	38.41 ± 0.70	52.90 ± 1.11
4.	400	56.88 ± 3.55	78.93 ± 4.13	58.62 ± 2.45	67.22 ± 0.83
5.	500	75.46 ± 2.58	90.27 ± 1.08	75.89 ± 7.89	7970 ± 2.81

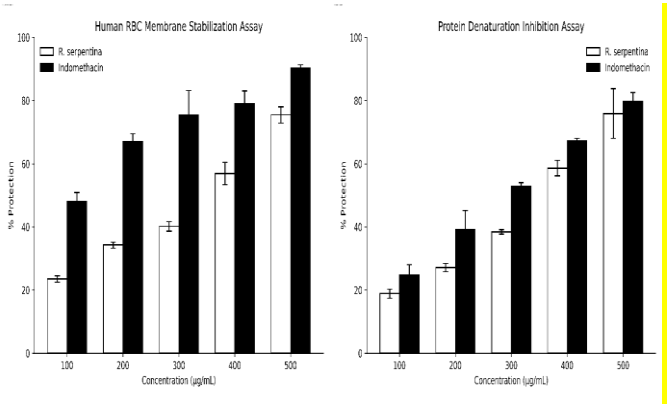


Figure-5 Represents the Comparison between indomethacin and methanolic extract of Rauwolfia *Serpentina* roots powder

Discussions

The present study demonstrates that the methanolic root extract of *Rauwolfia serpentina* possesses significant antioxidant and anti-inflammatory activities, which may be attributed to its diverse phytochemical constituents, including phenolic compounds, flavonoids, and bioactive metabolites identified through GC–MS analysis. The extract exhibited moderate DPPH radical scavenging activity, indicating its capacity to neutralize reactive oxygen species and reduce oxidative stress, a major contributor to chronic inflammatory and degenerative diseases. The presence of phenolic and flavonoid compounds is known to enhance antioxidant defense mechanisms through hydrogen donation and free radical stabilization.

Similar findings have been reported in several medicinal plants such as Curcuma longa, Withania somnifera, and Asparagus racemosus, where phenolic-rich extracts demonstrated strong antioxidant and anti-inflammatory effects through inhibition of oxidative stress-mediated pathways. The GC–MS analysis of *Rauwolfia serpentina* revealed compounds such as phenylacetic acid, palmitic acid, and isopropyl myristate, which have been associated with anti-inflammatory, antimicrobial, and cytoprotective activities. The extract also showed notable inhibition of COX-2 enzyme activity and significant protection against protein denaturation and erythrocyte membrane lysis, suggesting stabilization of cellular membranes and suppression of inflammatory mediator release. These mechanisms are comparable to those reported for other medicinal plants used in traditional medicine for treating inflammatory disorders. Furthermore, the concentration-dependent response observed in the HRBC membrane stabilization and protein denaturation assays support the therapeutic potential of the extract as a natural anti-inflammatory agent. Overall, the findings validate the traditional use of *Rauwolfia serpentina* and suggest that its phytochemical constituents may serve as promising candidates for the development of plant-based therapeutics against oxidative stress and inflammation-related diseases.

Conclusion

The present study demonstrates that the methanolic root extract of *Rauwolfia serpentina* possesses considerable antioxidant and anti-inflammatory activities, supported by its rich phytochemical composition. The extract exhibited effective free radical scavenging potential, significant COX-2 inhibitory activity, membrane stabilization, and protein denaturation inhibition in a dose-dependent manner. GC–MS analysis further revealed the presence of several bioactive compounds that may contribute to these

pharmacological effects. These findings scientifically validate the traditional medicinal use of *Rauwolfia serpentina* and suggest its potential as a natural source of therapeutic agents for the management of oxidative stress and inflammatory disorders.

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