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Phytochemical Characterization, Mineral Profiling, and *In Vitro* Assessment of the Antioxidant and Anti-Inflammatory Potential of *Strobilanthes pavala* (Roxb.) J.R.I. Wood Whole Plant Ethanolic Extract

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Abstract: The primary objective of this investigation was to systematically analyze the phytochemical constituents, structural functional groups, elemental distribution, and *in vitro* antioxidant and anti-inflammatory activities of the whole plant ethanolic extract of *S. pavala*. Qualitative phytochemical screening of the ethanolic extract of *S. pavala* (EESP) indicated a prominent presence of polyphenols, flavonoids, tannins, and terpenoids. FTIR analysis of the organic residues resolved distinctive structural functional groups, notably hydrogen-bonded hydroxyl networks, aliphatic chains and carboxylic/ester carbonyls aromatic skeletal vibration confirming a dense matrix of polar flavonoid glycosides and polyphenols. Quantitative ICP-MS analysis revealed a valuable accumulation of essential minerals, resolved in order of quantitative dominance as Potassium (17365.0±444.0 µg/g), Magnesium (4952.5 ±119.8 µg/g), Calcium (1257.0 ±10.5 µg/g), Sodium (226.5 ±2.0 µg/g), Iron (104.0 ±2.9 µg/g), Manganese (6.0 ±0.5 µg/g), Chromium (15.0 ±0.5 µg/g), Zinc (11.0 ±0.4 µg/g), Molybdenum (0.56 ±0.02 µg/g), Cobalt (0.12±0.01 µg/g) and Selenium (0.09 ±0.01 µg/g). The extract exhibited remarkable, concentration-dependent antioxidant capacity, yielding highly potent IC50 values of 55.40±0.56 µg/mL for DPPH and 50.08 ±0.15 µg/mL for NO scavenging. Furthermore, EESP demonstrated notable anti-inflammatory action by protecting GRBC membranes from thermal lysis with an IC50 of 175.08 ±0.93 µg/mL, and preventing bovine serum albumin denaturation with an IC50 of 188.99±6.16 µg/mL. The compiled evidence demonstrates that the whole plant of *S. pavala* constitutes a rich reservoir of bio-accessible essential macroelements and trace metals, confirming its therapeutic potential as a natural antioxidant and anti-inflammatory formulation.

Keywords: *Strobilanthes pavala; EESP; Cold Maceration; Phytochemical Screening; FTIR; ICP-MS; Anti-inflammatory; Membrane Stabilization; GRBC; Protein Denaturation Assay; Antioxidant; DPPH; SEM.*

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I. INTRODUCTION

The exploration of botanical resources for novel bioactive molecules continues to be a cornerstone of modern pharmacognosy and drug development. Within the Acanthaceae family, the genus *Strobilanthes* stands out as a highly diverse group of plants distributed extensively across the tropical zones of Asia, recognized for both their unique ecological life cycles and their historical applications in folklore medicine[1]. Recent molecular and taxonomic revisions have integrated several taxa from the morphologically similar genus *Hemigraphis* into *Strobilanthes* based on palynological, floral, and nuclear ribosomal DNA sequence comparisons[2]. Consequently, the shade-loving, decumbent herb traditionally known as *Hemigraphis atebrosa* (B.Heyne ex Roth) Nees has been taxonomically reassessed and validated under its accepted binomial name, *Strobilanthes pavala* (Roxb.) J.R.I. Wood[3]. This species is widely distributed across the Indian subcontinent, thriving on shaded forest floors, damp rock crevices, and understory ecosystems of the Western Ghats and Deccan Peninsula.

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Despite its geographical distribution, *S. pavala* remains a significantly under-researched botanical entity with a glaring scientific validation deficit. Ethnobotanical sources register this herb as a vital "medico-food" within various indigenous communities of India. For instance, tribal populations in Odisha utilize the fresh, nutrient-dense leaves as a wild leafy vegetable during monsoon seasons, while traditional healers in the Melghat and Baitul forest tracts prescribe whole-plant decoctions as systemic blood purifiers to resolve dermatological eruptions and chronic systemic heat[4]. Furthermore, tribal practices in Southern India employ its root juice as a specialized nasal drop to counter acute alcohol intoxication and leverage fresh leaf poultices to rapidly cool inflammatory skin lesions, insect bites, and minor burns[5]. In historical South Indian Siddha manuscripts, the plant is referred to as *"Vella-kanni"* (the white-eyed creeper) and is celebrated for its capacity to pacify aggravated *Pitta* dosha, demonstrating its deep-rooted therapeutic legacy.

Biomedical paradigms suggest that the pharmacological efficacy of *S. pavala* lies in its highly specialized secondary metabolites. Allied species within the subtribe *Strobilanthinae* are characterized by an abundance of anthocyanins (such as cyanidin-3-glucoside), flavones (such as luteolin), and diverse phenolic acids (such as caffeic and chlorogenic acids)[6]. These compounds function as powerful, synergistic biochemical buffers. For example, luteolin-rich fractions have been demonstrated to suppress the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), effectively halting the cascade of pro-inflammatory cytokines governed by the transcription factor NF-kappaβ[7]. Concurrently, anthocyanin molecules protect vulnerable cellular and vascular endothelial membranes from oxidative degeneration.

At the cellular level, pathogenic oxidative stress arises from an unchecked accumulation of reactive oxygen species (ROS) and reactive nitrogen species, such as peroxynitrite. These reactive intermediates trigger lipid peroxidation, cellular membrane lysis, and the rapid, thermal denaturation of vital structural proteins[8]. By protecting the physical integrity of membranes and preserving the folding states of proteins, botanical extracts can limit inflammatory tissue degradation. These protective cellular mechanisms can be modeled in-vitro using red blood cell membrane stabilization and albumin denaturation assays.

Importantly, the therapeutic value of a medicinal plant is determined not only by its organic molecules but also by its inorganic elemental profile. Essential macro-minerals (such as Potassium, Calcium, Magnesium, and Sodium) and trace elements (such as Iron, Zinc, Copper, Manganese, Chromium, Cobalt, Selenium, and Molybdenum) play critical roles in biological pathways. They act as osmotic regulators, structural components of plant cell walls, and mandatory functional cofactors for endogenous cellular antioxidant enzymes like Superoxide Dismutase, Catalase , and Glutathione Peroxidase [9]. To establish a rigorous safety and efficacy profile, chemical investigations must couple organic spectroscopy with highly sensitive inorganic profiling.

To address these parameters, this investigation presents a comprehensive, multi-dimensional study of *Strobilanthes pavala*. We detail the cold maceration extraction of its whole plant tissue using analytical-grade ethanol to yield the Ethanolic Extract of *Strobilanthes pavala* (EESP). This extract was systematically characterized using preliminary phytochemical screens, functional group fingerprinting via Fourier-Transform Infrared (FTIR) spectroscopy, and absolute multi-element quantification via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Finally, we validate its bioprotective efficacy through *in vitro* goat red blood cell (GRBC) membrane stabilization, bovine serum albumin (BSA) denaturation inhibition, and free-radical (DPPH and NO) scavenging assays.

II. MATERIALS AND METHODS

- *Collection and Authentication of Plant Sample*

Fresh, healthy plant material of Strobilanthes pavala (Roxb.) J.R.I. Wood was harvested from Keoradanga, West Bengal, India. The collection site is geographically situated at latitude 22.3673240 N and longitude 88.3078990 E, at an average baseline elevation of approximately 9 m above sea level. Taxonomic identification and authentication of the collected plant specimens were performed by a botanist at the Herbarium of the Botanical Survey of India (BSI), Central National Herbarium, Shibpur, Howrah, West Bengal. A representative voucher specimen was processed and formally cataloged under registration index BSI-SP-2026/01 for future reference as shown Fig 1.

The collected whole plant material was washed thoroughly under running tap water to eliminate soil, dust, and particulate matter, followed by a secondary rinse with distilled water. The specimens were then shade-dried at an ambient room temperature of 28 ±2 ^{0}C for 14 days. Once completely dehydrated, the whole plant tissue was ground into a coarse powder using an electric laboratory grinder, sieved to obtain uniform particles, and stored in hermetically sealed glass container systems to avoid thermal or enzymatic decomposition of volatile or vulnerable bioactive constituents[10].

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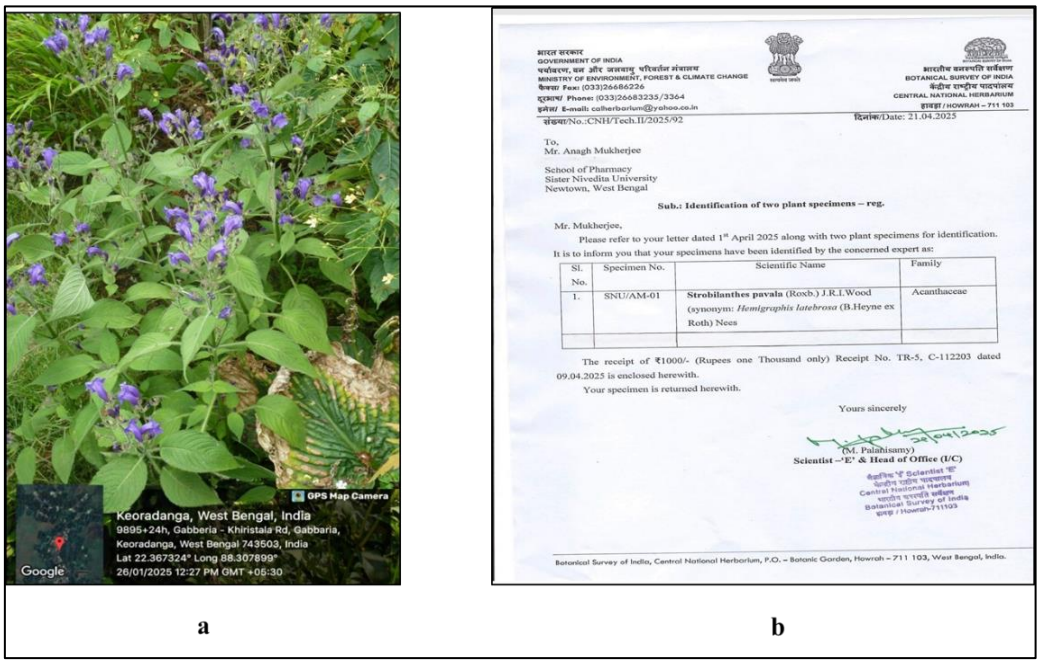

Fig 1 (A) Plant Collection, (B) Plant Authentication

➢ *Preparation of Crude Extract*

To preserve delicate, thermolabile bioactive secondary metabolites and avoid potential heat-induced chemical alterations, the crude extract was prepared using a simple cold maceration method[11]. A portion of 100 g of the dehydrated whole plant powder was placed in a sterilized, glass-stoppered container and immersed in analytical-grade ethanol at a solvent-to-sample ratio of 5:1 v/w. The mixture was allowed to stand at an ambient laboratory temperature of 28 ±2 ^{0}C for a duration of 72 hours (3 days) with continuous or periodic mechanical agitation to facilitate the complete diffusion and mass transfer of intracellular components into the solvent. Following the 72 hour maceration period, the mixture was filtered first through double-layered muslin cloth to remove coarse particulate matter, and subsequently clarified using Whatman No. 1 filter paper under vacuum. The resulting clear ethanolic filtrate was concentrated using a rotary evaporator operating under reduced pressure at a controlled temperature of 40^{0}C to prevent structural degradation of the extracted molecules. This process yielded a semi-solid, dark-green residue (11.8%w/w). This dry ethanolic extract of S. pavala (EESP) was stored in a vacuum desiccator at 4 ^{0}C until further qualitative, structural, and pharmacological testing[12].

➢ *Preliminary Phytochemical Screening*

Qualitative chemical evaluations of EESP were conducted using established diagnostic methodologies to verify the presence of major secondary metabolite groups including alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins[13].

➢ *Fourier-Transform Infrared (FTIR) Spectroscopy*

For functional group mapping, dried EESP was analyzed using an FTIR spectrophotometer (Bruker Alpha II, Germany). Approximately 2 mg of the dry macerated extract was blended thoroughly with 200 mg of dry, spectroscopic-grade potassium bromide (KBr) powder and compressed in a hydraulic press at 10 tons of pressure to form a translucent, thin disc. The KBr disc was placed in the sample chamber and scanned across the mid-infrared region (4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1} for 32 successive scans under a nitrogen atmosphere[14].

➢ *Inductively Coupled Plasma Mass Spectrometry (ICP-MS)*

The elemental composition and trace metal profile of the whole plant powder were determined using an ICP-MS instrument (Agilent 7800 ICP-MS, USA) following microwave-assisted wet digestion using 6 mL of ultrapure nitric acid and 2 mL of hydrogen peroxide[15].

➢ *In Vitro Anti-Inflammatory Activity*

Goat red blood cell membrane stabilization was tested using physiological Alsever's solution suspension against heat-induced lysis at 560C for 30 minutes, measuring hemoglobin optical densities at 560 nm[16]. BSA protection was evaluated spectrophotometrically at 660 nm against water-bath thermal unfolding configurations at 700C for 5 minutes.

III. RESULTS AND DISCUSSION

Preliminary Phytochemical Screening Qualitative analysis of EESP revealed abundant levels of crucial defense metabolites, as tabulated below.

➢ *FTIR Spectroscopy Analysis*

The experimental FTIR spectrum of EESP displayed in Fig. 2, multiple highly resolved absorbance valleys as displayed in Table 2, confirming a rich density of polar polyphenolics and flavonoid glycosides.

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➢ *ICP-MS Elemental Analysis*

The quantitative mineral profile and trace metal concentrations of the dried S. pavala whole plant tissue were determined using microwave-assisted digestion followed by ICP-MS analysis. Quantitative absolute element concentrations obtained from ICP-MS digestion run models are compiled in Table 3. The elemental analysis demonstrates that S. pavala accumulates high levels of essential macrominerals. Potassium was found to be the most abundant mineral constituent, which is consistent with the standard physiological profile of terrestrial plants, where potassium acts as the primary osmotic driver, cytosolic pH buffer, and catalytic activator of essential metabolic enzymes. This is closely followed by Magnesium, which acts as the core coordinating element of the chlorophyll porphyrin ring and functions as an obligatory cofactor for kinases and ATP-utilizing proteins in photosynthetic cells. Calcium provides structural integrity to the cell walls by cross-linking pectin polysaccharides in the middle lamella, and acts as a central second messenger in stress-responsive signaling pathways. Sodium is present in moderate amounts, assisting in cell turgor and electrochemical potential maintenance. Among the transition metals and trace elements, Iron is highly represented. It serves a vital role as a structural cofactor in heme and iron-sulfur cluster proteins (including ferredoxin, cytochromes, catalase, and peroxidase), which are essential for electron transport during photosynthesis and respiration. Manganese and Zinc are essential for photosynthetic oxygen evolution and zinc-finger protein folding configurations, respectively. Additionally, Zinc serves as a critical cofactor for Cu/Zn-SOD enzymes which clear superoxide radicals from cell structures. The analytical sequence also quantified vital ultra-trace elements: Chromium, Molybdenum, Cobalt and Selenium. Chromium has been widely documented to enhance insulin receptor sensitivity and optimize glucose utilization in mammals, which may support the traditional use of this botanical as a medicinal food. Molybdenum serves as an indispensable coordinator in nitrate reductase and xanthine oxidase systems, while Cobalt is required for the synthesis of cobalamin complexes. Selenium, though a minor trace constituent, is integrated directly into selenoproteins like Glutathione Peroxidase, reinforcing the overall cellular defense of the consumer. Critically, these elements are present in concentrations well below the toxicity thresholds established by the WHO, validating the safe mineral profile of this botanical species.

➢ *D. In Vitro Anti-Inflammatory Activity*

The anti-inflammatory efficacy of EESP was evaluated through its ability to protect goat red blood cell (GRBC) membranes against thermal lysis and to suppress the denaturation of bovine serum albumin (BSA). To present these findings with optimal comparative clarity, both assay configurations have been consolidated into a single statistical dataset (Table 4) sharing identical concentration coordinates (50 to 800 µg/mL) as shown in Fig.3.

During physical trauma or pathogen-induced chronic inflammation, lysosomal membranes undergo physical disruption, prompting the uncontrolled release of highly destructive, autolytic lysosomal enzymes into surrounding extracellular tissue. Because the lysosomal membrane corresponds structurally and functionally to the fragile phospholipid bilayer of goat erythrocytes, stabilization of the GRBC membrane under thermal stress serves as a valuable surrogate system for determining *in vitro* anti-inflammatory capabilities[17].

The experimental values show that EESP exhibits strong, dose-dependent GRBC membrane protection across the tested concentrations. These values yielded an *IC50* value of 175.08 ± 0.93 µg/mL for EESP. In comparison, the reference NSAID Diclofenac Sodium yielded a corresponding *IC50* of 97.18 ± 0.24 µg/mL. This robust membrane stabilizing capacity indicates that the secondary metabolites in *S. pavala* (such as the flavonoid glycosides and tannins identified via qualitative and FTIR screening) interact with membrane-anchored surface proteins, effectively altering surface charge density and stabilizing the physical architecture of the lipid bilayer against thermal lysis. Concurrently, systemic tissue protein denaturation is heavily implicated in the pathogenesis of chronic inflammatory conditions, including rheumatoid arthritis. Thermal denaturation of albumin is a well-established model for investigating the modulation of protein folding/stabilization by bioactive ligands under inflammatory conditions, where structural modifications in tissue autoantigens can trigger a self-perpetuating autoimmune cascade. As illustrated by the experimental results EESP significantly prevented the thermal denaturation of bovine serum albumin (BSA) in a dose-dependent manner. These data points yielded a highly potent experimental *IC50* of 188.99 ± 6.16 µg/mL for EESP. In comparison, standard Diclofenac Sodium exhibited an *IC50* of 99.58 ±0.18 µg/mL.

The molecular mechanism behind this protein stabilization is likely related to the high content of polyphenolic substances and flavonoids resolved in the extract's FTIR spectrum (such as the broad phenolic hydroxyl group at 3403.48 cm^{-1}, conjugated aromatic skeletal stretch at 1622.50 cm^{-1} and glycosidic ether bands at. These compounds easily access and secure vulnerable electrostatic and hydrophobic centers in albumin structure. This dual capacity to protect cellular membranes and prevent protein folding disruptions (as consolidated in Table 4) provides strong chemical and physiological evidence supporting the traditional utilization of *S. pavala* as a protective therapeutic agent.

➢ *In Vitro Antioxidant Activity*

The free-radical scavenging efficiency (DPPH and NO assays) has been compiled in Table 5. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay serves as a classical system to measure the lipid-radical scavenging kinetics of botanical compounds. The assay relies on the chemical reduction of the stable, deep-violet DPPH nitrogen radical to its corresponding yellow hydrazine derivative (diphenylpicrylhydrazine) via electron or hydrogen atom transfer from the antioxidant compounds present in the extract[18]. As illustrated by the experimental results in Table 5, the whole plant extract of *S. pavala* (EESP) displayed a powerful and highly consistent, dose-dependent radical-

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clearing capability. Based on these triplicate runs, the *IC50* value of EESP was resolved to 55.40 ± 0.56 µg/mL showing an excellent biological efficiency that compares favorably with the pure reference standard Ascorbic Acid 35.26 ±0.83 µg/mL.

In parallel, the nitric oxide (NO) scavenging assay demonstrated highly compelling inhibitory dynamics. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which rapidly reacts with oxygen to yield highly reactive, cytotoxic nitrite radicals. When introduced to this system, EESP exhibited significant, concentration-dependent NO radical scavenging activity. This sharp initial kinetics curve yielded a highly competitive *IC50* value of 50.08 ± 0.15 µg/mL for EESP, which is remarkably close to that of the pure standard Ascorbic Acid 27.67 ±0.48 µg/mL as shown in Fig.4.

Table 1 Phytochemical Screening of The Whole Plant Extract of *S. Pavala*

Phytochemical Group	Chemical Test Conducted	Results
Alkaloids	Mayer's Test / Wagner's Test	Positive
Flavonoids	Shinoda Test	Positive
Phenols	Ferric Chloride Test	Positive
Tannins	Gelatin Test	Positive
Terpenoids	Salkowski Test	Positive
Saponins	Froth Test	Negative

Table 2 FTIR Spectral Band Assignments for *S. Pavala* Extract

Observed Wavenumber (cm^{-1})	Peak Intensity & Shape	Vibrational Assignment	Correlating Phytoconstituent Class
3403.48	Strong, broad valley	Hydrogen-bonded O-H stretching	Phenolics, alcohols, flavones, water
2924.28	Strong, sharp plunge	Asymmetric C-H stretching	Aliphatic chains, waxes, terpenoids
2852.81	Medium, sharp plunge	Symmetric C-H stretching	Saturated structures, lipids
1703.96	Medium, shoulder	C=O stretching vibration	Carbonyls of organic acids, esters
1622.50	Strong, sharp plunge	Conjugated C=O or skeletal C=C stretch	Flavonoid backbone, anthocyanins
1056.16	Strong, broad valley	C-O stretching / C-O-C glycoside stretch	Glycosidic linkages, primary alcohols

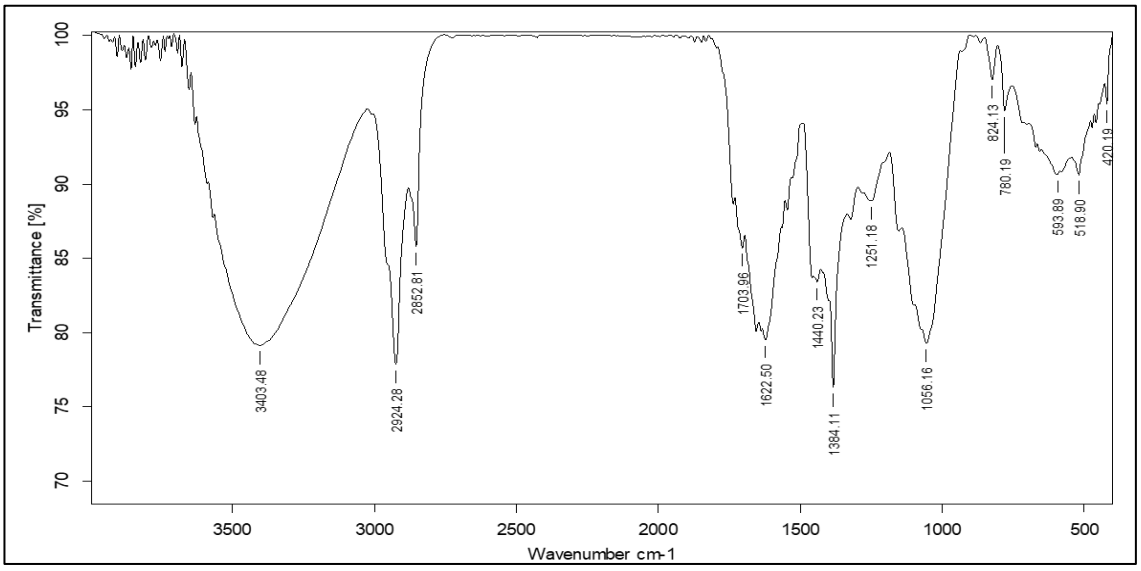

Fig 2 Traced Experimental FTIR Spectrum of The Whole Plant Ethanolic Extract of *S. Pavala* Demonstrating Distinct Structural Bands.

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Table 3 Elemental Analysis of *S. Pavala* Whole Plant Via ICP-MS

Element Classified	Analytical Isotopes (m/z)	Concentration (µg/g dry weight)
Potassium (K)	^{39}K	17365.0 ± 444.0 (Range: 16596.0–18134.0)
Magnesium (Mg)	^{24}Mg	4952.5 ± 119.8 (Range: 4745.0–5160.0)
Calcium (Ca)	^{44}Ca	1257.0 ± 10.5
Sodium (Na)	^{23}Na	226.5 ± 2.0 (Range: 223.0–230.0)
Iron (Fe)	^{56}Fe	104.0 ± 2.9
Zinc (Zn)	^{66}Zn	11.0 ±0.4
Selenium (Se)	^{82}Se	0.09 ±0.01

Table 4 Consolidated *In Vitro* Anti-Inflammatory Activities of EESP

Concentration (µg/mL)	GRBC Protection (%) (EESP)	GRBC Protection (%) (Diclofenac)	BSA Inhibition (%) (EESP)	BSA Inhibition (%) (Diclofenac)
50.00	14.47 ± 0.73	36.05 ±0.70	12.09 ±0.16	21.58 ±1.76
100.00	40.11 ± 0.67	66.15 ±1.02	37.27 ±0.51	54.00 ±1.76
200.00	49.12 ± 0.33	78.99 ±0.87	48.35 ±0.44	66.34 ±2.85
400.00	58.67 ± 2.67	84.95 ±0.73	51.60 ±0.74	73.78 ±1.53
800.00	67.03 ± 0.67	90.86 ±0.85	63.59 ±1.11	83.89 ±1.76
IC50 (µg/mL)	175.08 ± 0.93	97.18 ± 0.24	188.99 ± 6.16	99.58 ±0.18

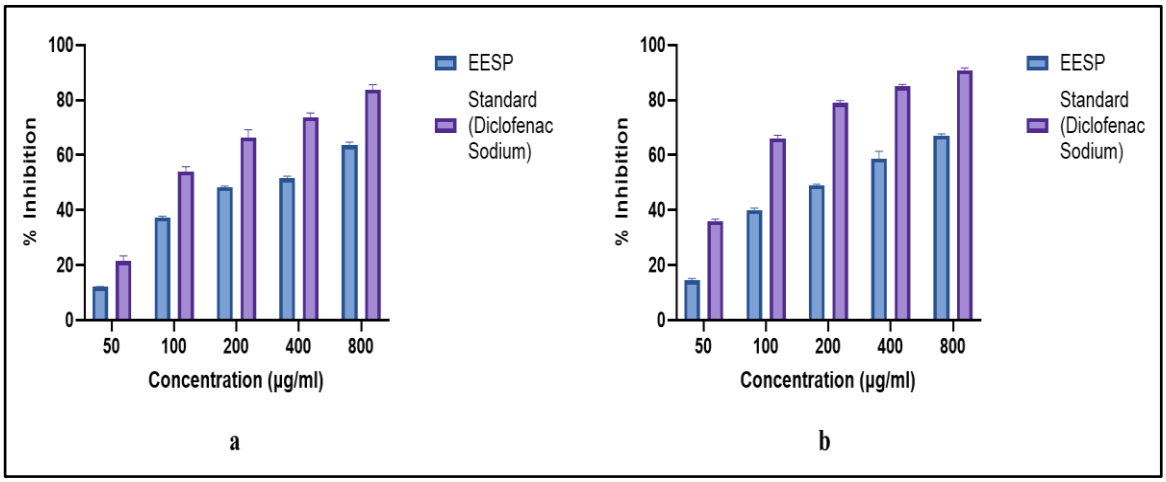

Fig 3 In-Vitro Anti-Inflammatory Activity (A) Protein Denaturation, (B) Membrane Stabilization Of Ethanol Extract Of *Strobilanthes Pavala* (EESP). Values Are Expressed As Mean ± SEM (N = 3)

Table 5 *In Vitro* Antioxidant Activities of EESP

Concentration (µg/mL)	DPPH Scavenging (%) (EESP)	DPPH Scavenging (%) (Ascorbic Acid)	NO Scavenging (%) (EESP)	NO Scavenging (%) (Ascorbic Acid)
25.00	14.37 ± 0.32	40.73 ±0.51	22.40 ±0.87	46.83 ±0.42
50.00	38.44 ± 0.29	62.14 ±0.38	49.52 ±0.87	65.61 ±0.19
100.00	44.68 ± 0.66	72.18 ±0.99	58.04 ±0.58	74.99 ±0.17
200.00	51.74 ±0.37	81.26 ±0.52	67.36 ±0.88	85.33 ±0.33
400.00	55.38 ±0.31	85.66 ±0.30	68.19 ±0.61	87.67 ±0.33
IC50 (µg/mL)	55.40 ± 0.56	35.26 ±0.83	50.08 ± 0.15	27.67 ±0.48

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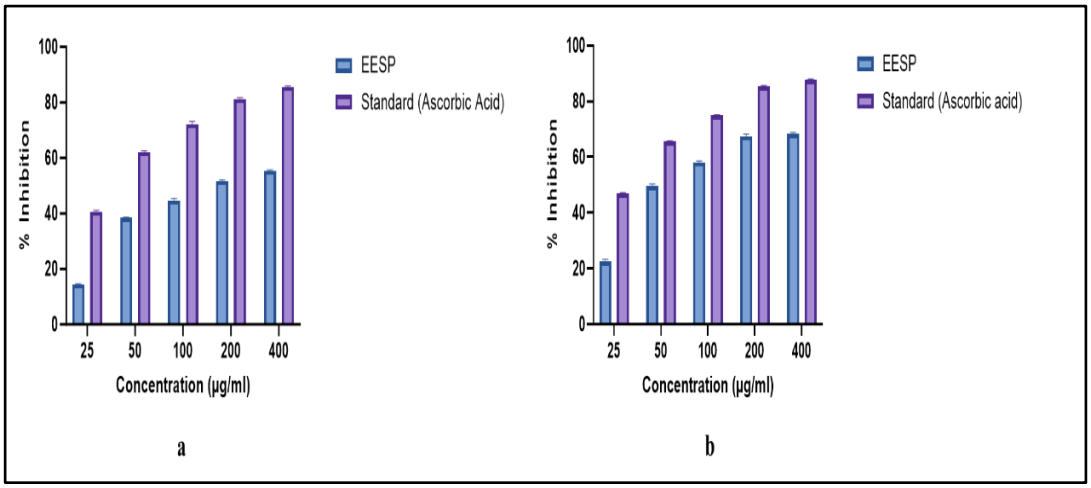

Fig 4 In-Vitro Anti-Oxidant Activity (A) A, A-Diphenyl-B-Picryl-Hydrazyl (DPPH) Radical Scavenging Assay, (B) Nitric Oxide (NO) Radical Scavenging Assay of Ethanol Extract of *Strobilanthes Pavala* (EESP). Values Are Expressed As Mean ± SEM (N = 3)

IV. CONCLUSION

This study offers the first comprehensive, scientific characterization of the phytochemical profile, functional groups, elemental composition, and *in vitro* pharmacological activities of *Strobilanthes pavala* whole plant ethanolic extract. In conclusion, these findings validate the ethnobotanical value of *S. pavala* as a natural source of bio-active compounds with potential applications in managing oxidative stress and chronic inflammatory conditions.

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