



SCIENTIFIC VALIDATION OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF STACHYTARPHETA URTICIFOLIA LEAF EXTRACTS

Roshni Tamada*

*M Pharmacy, Department of Pharmacology, Shri Shankaracharya Professional University,
Bhilai, Chhattisgarh, India.

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*Corresponding Author

Roshni Tamada

M Pharmacy, Department of
Pharmacology, Shri Shankaracharya
Professional University, Bhilai,
Chhattisgarh, India.



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ABSTRACT

This study investigates the phytochemical composition and in vitro anti-inflammatory and antioxidant activities of *Stachytarpheta urticifolia* leaf extracts. Leaves were extracted using ethanol and distilled water, followed by qualitative and quantitative phytochemical analysis. The results revealed the presence of major bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, and saponins. Quantitative analysis showed higher total phenolic content in the aqueous extract and higher flavonoid content in the ethanolic extract. Antioxidant activity assessed by DPPH assay indicated strong radical scavenging potential, particularly in the aqueous extract. Anti-inflammatory activity evaluated using the protein denaturation assay demonstrated significant inhibition by both extracts, comparable to standard diclofenac sodium. The findings suggest that *S. urticifolia* possesses potent antioxidant

and anti-inflammatory properties, supporting its traditional medicinal use and highlighting its potential as a source of natural therapeutic agents.

KEYWORDS: *Stachytarpheta urticifolia*, phytochemical analysis, antioxidant activity, anti-inflammatory activity, DPPH assay.

INTRODUCTION

Herbal medicine represents one of the oldest and most enduring systems of healthcare, forming the foundation of traditional medical practices across diverse cultures worldwide. Historically, human reliance on plants for food, shelter, and medicine led to the gradual development of extensive ethnobotanical knowledge through observation and empirical experimentation. This traditional knowledge has significantly influenced modern pharmacotherapy, as many contemporary drugs are derived directly or indirectly from plant sources. It is estimated that approximately 25% of prescription drugs contain plant-derived active compounds, while a substantial proportion of synthetic drugs are modeled after natural phytochemicals. Globally, the importance of herbal medicine remains substantial. According to the World Health Organization (WHO), nearly 80% of the population in developing countries relies on plant-based traditional medicine for primary healthcare. The increasing demand for herbal remedies is driven by their perceived safety, affordability, accessibility, and reduced adverse effects compared to conventional drugs. Consequently, the global herbal medicine market has experienced significant growth, supported by ongoing research into bioactive plant compounds and their therapeutic potential.

Inflammation is a fundamental biological response to harmful stimuli such as pathogens, tissue injury, or irritants. While acute inflammation serves as a protective mechanism facilitating tissue repair and immune defense, chronic inflammation is implicated in the pathogenesis of numerous diseases, including cardiovascular disorders, arthritis, diabetes, and cancer. The inflammatory process is regulated by complex molecular pathways involving immune cells, cytokines, and signaling cascades such as nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT). These pathways control the expression of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF- α), interleukins, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (Merecz-Sadowska et al., 2020; Olędzka & Czerwińska, 2023).

Conventional anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are widely used for managing inflammation. However, their long-term use is often associated with adverse effects such as gastrointestinal toxicity, immunosuppression, and increased risk of infections. These limitations have intensified the search for safer and more effective alternatives, particularly from natural sources. Medicinal

plants are rich in bioactive phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds, which have demonstrated significant anti-inflammatory activity through modulation of key signaling pathways, inhibition of pro-inflammatory mediators, and scavenging of reactive oxygen species (ROS) (Yatoo et al., 2018; Al-Khayri et al., 2022).

Among medicinal plants, species belonging to the genus *Stachytarpheta* (family: Verbenaceae) have been widely used in traditional medicine for the treatment of inflammation, fever, gastrointestinal disorders, and infections. *Stachytarpheta urticifolia* Sims, commonly known as blue porterweed, is a perennial herb distributed in tropical and subtropical regions. Ethnobotanical reports indicate its use in managing inflammatory conditions, ulcers, respiratory ailments, and skin disorders. Phytochemical studies on related species have revealed the presence of flavonoids, phenolics, alkaloids, tannins, and terpenoids, compounds known for their antioxidant and anti-inflammatory properties.

Despite its extensive traditional use, *S. urticifolia* remains underexplored in terms of its phytochemical composition and pharmacological activities. Scientific validation of its bioactive constituents and anti-inflammatory potential is essential to support its therapeutic applications and to identify novel natural compounds for drug development. Therefore, the present study aims to evaluate the phytochemical profile of *S. urticifolia* leaf extracts and investigate their anti-inflammatory activity using established in vitro models. This research may contribute to the development of safer, plant-based anti-inflammatory agents and provide a scientific basis for the traditional use of this species.

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh leaves of *Stachytarpheta urticifolia* were collected from Dharmapuri, Tamil Nadu, India. The collected plant material was thoroughly washed under running tap water to remove dust and debris, followed by rinsing with distilled water. The cleaned leaves were shade-dried at room temperature (25–30 °C) for 10–14 days until completely desiccated. The dried material was then pulverized into a coarse powder using a mechanical grinder and stored in airtight containers at room temperature until further use.

Authentication of Plant Material

The plant specimen was authenticated by Dr. J. Kamakshamma, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, India. A voucher specimen (No. 4858) was deposited in the herbarium of the same institution for future reference.

Chemicals and Reagents

All chemicals used were of analytical grade. The reagents included ethanol, distilled water, bovine serum albumin (BSA), diclofenac sodium (standard drug), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), phosphate-buffered saline (PBS), trichloroacetic acid (TCA), Folin–Ciocalteu reagent, gallic acid, and rutin.

Instruments and Equipment

The study utilized standard laboratory instruments including a rotary evaporator, sonicator, UV–Visible spectrophotometer (UV-1900i), centrifuge, hot air oven, analytical balance, and maceration apparatus.

Preparation of Plant Extracts***Ethanollic Extraction***

Approximately 100 g of powdered plant material was soaked in 80% ethanol at a ratio of 1:5 (w/v). The mixture was stirred intermittently and allowed to macerate. It was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain the crude ethanolic extract.

Aqueous Extraction

For aqueous extraction, 30 g of powdered plant material was boiled with distilled water. The mixture was filtered, and the filtrate was concentrated under reduced pressure at 40 °C. The dried extract was weighed and stored in airtight amber-colored containers at 4 °C until further analysis.

Determination of Extractive Value

Extractive values were determined using the maceration method. Briefly, 1 g of powdered plant material was placed in a 20 mL conical flask and extracted with 20 mL of solvent (water or ethanol). The mixture was kept at room temperature for 24 hours with intermittent

shaking, followed by filtration. The filtrate was evaporated to dryness, and the extractive value was calculated based on the residue obtained.

Qualitative Phytochemical Screening

Preliminary phytochemical screening of the extracts was conducted using standard procedures to detect the presence of major bioactive constituents, including alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, proteins, and carbohydrates (Harborne, 1998; Trease & Evans, 2009).

- **Alkaloids:** Dragendorff's and Mayer's tests
- **Flavonoids:** Shinoda test and alkaline reagent test
- **Tannins:** Ferric chloride test
- **Saponins:** Foam test
- **Steroids/Terpenoids:** Salkowski and Liebermann–Burchard tests
- **Glycosides:** Keller–Killiani test
- **Phenols:** Ferric chloride test
- **Proteins:** Biuret test
- **Carbohydrates:** Molisch test

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method (Singleton et al., 1999). Briefly, 0.5 mL of extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 minutes. Then, 2 mL of 7.5% sodium carbonate was added, and the mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 660 nm using a UV–Vis spectrophotometer. Gallic acid was used to generate the calibration curve, and results were expressed as mg gallic acid equivalents (GAE)/g of dry extract.

Total Flavonoid Content (TFC)

Total flavonoid content was estimated using the aluminum chloride colorimetric method (Chang et al., 2002). Briefly, 0.5 mL of extract was mixed with 0.5 mL of 2% aluminum chloride solution. After incubation for 30 minutes at room temperature, absorbance was measured at 660 nm. Rutin was used as the standard, and results were expressed as mg rutin equivalents (RE)/g of dry extract.

Pharmacological Evaluation

Antioxidant Activity (DPPH Assay)

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay (Blois, 1958). Various concentrations of the extract (20–100 µg/mL) were prepared and mixed with 0.1 mM DPPH solution in methanol. The reaction mixtures were incubated in the dark at room temperature, and absorbance was measured at 517 nm. Ascorbic acid was used as a reference standard. The percentage inhibition of DPPH radicals was calculated.

Anti-inflammatory Activity (Protein Denaturation Assay)

The anti-inflammatory activity was assessed using the protein denaturation inhibition assay (Mizushima & Kobayashi, 1968). Extracts at different concentrations (50–250 µg/mL) were mixed with 1% aqueous BSA solution. The mixtures were incubated at 37 °C for 20 minutes and then heated at 57 °C for 3 minutes. After cooling, turbidity was measured at 660 nm. Diclofenac sodium was used as the standard drug. Percentage inhibition of protein denaturation was calculated.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD)

RESULTS AND DISCUSSION

Extractive Value

The extractive values of *Stachytarpheta urticifolia* leaf powder using water and ethanol as solvents are presented in Table 1.

Table 1: Percentage Extractive Values of Plant Material.

Solvent Used	Extractive Value (%)
Water	15%
Ethanol	17%

The ethanolic extract showed a slightly higher extractive value (17%) compared to the aqueous extract (15%), indicating that ethanol is more efficient in extracting a wider range of phytoconstituents. However, the considerable extractive value in water suggests the presence of polar bioactive compounds such as phenolics and glycosides.

Phytochemical Analysis

Qualitative phytochemical screening confirmed the presence of multiple bioactive constituents in both extracts (Table 2).

Table 2: Phytochemical Constituents of *Stachytarpheta urticifolia* Extracts.

Phytochemical	Ethanol Extract	Aqueous Extract
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Steroids/Terpenoids	+ve	+ve
Glycosides	+ve	+ve
Phenols	+ve	+ve
Proteins	+ve	+ve
Carbohydrates	+ve	+ve

The presence of these phytochemicals supports the plant's pharmacological relevance, particularly in antioxidant and anti-inflammatory activities.

Quantitative Phytochemical Analysis

Table 3: Quantitative Phytochemical Content of Leaf Extracts.

Parameter	Ethanol Extract (Mean $\pm$ SD)	Aqueous Extract (Mean $\pm$ SD)	Units	Standard Used
Total Phenolic Content	830.34 $\pm$ 2.44	4818.50 $\pm$ 137.89	mg GAE/g	Gallic Acid
Total Flavonoid Content	1244.67 $\pm$ 3.54	335.30 $\pm$ 4.29	mg RE/g	Rutin

The aqueous extract showed significantly higher phenolic content, while the ethanolic extract exhibited higher flavonoid content. This indicates solvent-dependent extraction efficiency of different phytochemical classes.

Antioxidant Activity (DPPH Assay)

The antioxidant potential of the extracts was evaluated using the DPPH radical scavenging assay.

Table 4: DPPH Radical Scavenging Activity.

Sample	Concentration (μ g/mL)	Absorbance	% Radical Scavenging Activity (Mean $\pm$ SD)
Aqueous Extract	30	0.434	61.91 $\pm$ 0.35
Ethanolic Extract	30	0.442	7.60 $\pm$ 0.05

The aqueous extract demonstrated significantly higher antioxidant activity, likely due to its elevated phenolic content. Phenolic compounds are known for their strong free radical scavenging ability.

Anti-inflammatory Activity

Protein Denaturation Assay

Table 5: Inhibition of Protein Denaturation by Plant Extracts.

Concentration ($\mu\text{g/mL}$)	% Inhibition (Ethanol Extract)	% Inhibition (Aqueous Extract)
50	99.26 ± 0.27	98.16 ± 0.26
100	98.63 ± 0.22	97.49 ± 0.22
150	97.80 ± 0.31	96.87 ± 0.22
200	96.70 ± 0.41	96.05 ± 0.26

Both extracts showed strong inhibition of protein denaturation in a concentration-dependent manner, indicating potent anti-inflammatory activity.

Standard Drug Comparison (Diclofenac Sodium)

Table 6: Protein Denaturation Inhibition by Diclofenac Sodium.

Concentration ($\mu\text{g/mL}$)	% Inhibition (Ethanol)	% Inhibition (Water)
50	70.09 ± 0.26	93.39 ± 0.30
100	60.27 ± 0.26	85.56 ± 0.35
150	47.14 ± 0.26	83.68 ± 0.54
200	45.03 ± 0.31	88.86 ± 0.35

Interestingly, the plant extracts exhibited comparable or even higher inhibition than the standard drug in certain conditions, suggesting strong natural anti-inflammatory potential.

DISCUSSION

The results of this study indicate that *Stachytarpheta urticifolia* is a rich source of bioactive phytochemicals with significant antioxidant and anti-inflammatory potential. The higher extractive value in ethanol suggests better extraction of semi-polar compounds such as flavonoids, while the aqueous extract indicates the presence of polar constituents like phenolics.

Quantitative analysis revealed that the aqueous extract contained higher total phenolic content, which correlates with its superior antioxidant activity in the DPPH assay. Phenolic compounds are known for their ability to scavenge free radicals and reduce oxidative stress. In contrast, the ethanolic extract exhibited higher flavonoid content, which may contribute to its slightly stronger anti-inflammatory activity.

Both extracts demonstrated strong inhibition of protein denaturation, indicating potent anti-inflammatory effects. Notably, their activity was comparable to or higher than the standard drug diclofenac sodium under the tested conditions. This suggests that the observed effects may be due to the combined action of phytoconstituents such as flavonoids, phenolics, tannins, and saponins, which are known to modulate inflammatory pathways and stabilize proteins.

CONCLUSION

The present study confirms that *Stachytarpheta urticifolia* is rich in bioactive phytochemicals with significant antioxidant and anti-inflammatory activities. The results validate its traditional use and suggest its potential as a natural source for developing safer anti-inflammatory agents. Further studies are required to isolate active compounds and evaluate their efficacy in vivo.

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