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Research

Phytochemical Screening, HPTLC Standardization and *in-vitro* Antioxidant Activity of Ethanolic Extract of *Stachytarpheta Urticifolia* Leaves

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Abstract:
The ethanolic leaf extract of *Stachytarpheta urticifolia* demonstrated significant in vitro antioxidant activity, which may be attributed to its rich phenolic and flavonoid content, particularly quercetin. The findings support the traditional use of the plant and suggest that it may serve as a promising natural source of antioxidant phytoconstituents for further pharmacological investigation. Phytochemical screening revealed the presence of flavonoids, polyphenols, alkaloids, tannins, triterpenoids, saponins, carbohydrates, and amino acids in the ethanolic extract. Among all extracts, the ethanolic extract showed the highest percentage yield (26.63% w/w) and the highest total phenolic content (254.66 ± 19.55 mg QE/g extract). HPTLC fingerprinting of the ethanolic extract revealed 11 peaks, with a major peak at Rf 0.429 corresponding to quercetin, confirming its presence in the extract. The quercetin content was estimated to be approximately 0.214 mg/g of extract. In antioxidant studies, the ethanolic extract exhibited concentration-dependent activity in all assays. At 50 µg/mL, the extract showed 89.11 ± 3.15% inhibition in the superoxide radical scavenging assay and 89.26 ± 2.17% inhibition in the hydroxyl radical scavenging assay. In the lipid peroxidation assay, the extract showed 94.69 ± 3.12% inhibition at 250 µg/mL, which was higher than the standard.

Keywords: *Stachytarpheta urticifolia*; antioxidant activity; HPTLC; quercetin; total phenolic content; phytochemical screening; lipid peroxidation

1. INTRODUCTION

Medicinal plants have played a central role in traditional systems of medicine and continue to be an important source of therapeutic agents in modern drug discovery. The increasing interest in plant-derived medicines is largely attributed to their wide spectrum of pharmacological activities, structural diversity of phytoconstituents, and comparatively lower incidence of adverse effects when compared with many synthetic drugs. Among the various pharmacological properties of medicinal plants, antioxidant activity has received considerable scientific attention due to the critical role of oxidative stress in the pathogenesis of several chronic diseases.

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, and hydrogen peroxide are continuously generated in the body as by-products of normal metabolic processes. Under physiological conditions, these reactive species are neutralized by endogenous antioxidant defense systems. However, excessive production of ROS or depletion of endogenous antioxidants results in oxidative stress, leading to lipid peroxidation, protein oxidation, DNA damage, and cellular injury. Oxidative stress has been implicated in the development of cardiovascular diseases, neurodegenerative disorders, inflammatory conditions, diabetes mellitus, aging, and cancer. Therefore, identification of natural antioxidants from medicinal plants has become an important research area.

Stachytarpheta urticifolia Sims, belonging to the family Verbenaceae, is a perennial herb commonly known as blue snakeweed or nettle leaf velvet berry. It is widely distributed in tropical and subtropical regions and has been used traditionally for the treatment of fever, rheumatic inflammation, ulcers, venereal diseases, diarrhea, dysentery, and stomach disorders. Previous phytochemical investigations of this plant have indicated the presence of saponins, flavonoids, terpenoids, steroids, glycosides, alkaloids, tannins, and phenolic compounds. Some studies have also reported antioxidant and moderate cytotoxic activity in different extracts of the plant. However, comprehensive scientific evaluation of the ethanolic leaf extract with respect to its phytochemical profile, HPTLC standardization, and in vitro antioxidant activity remains limited.

Hence, the present study was designed to investigate the phytochemical constituents, total phenolic content, HPTLC fingerprint profile, and in vitro antioxidant potential of the ethanolic leaf extract of *Stachytarpheta urticifolia* leaves using superoxide radical scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibition assays.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh leaves of *Stachytarpheta urticifolia* were collected from Nellore, Andhra Pradesh, India, during February 2025. The plant material was authenticated using standard taxonomical references and by a qualified botanist/pharmacognosist. The collected leaves were washed, shade-dried at room temperature, powdered using a mechanical grinder, and stored in airtight containers for further use.

2.2 Preparation of Extracts

Approximately 1 kg of the powdered leaf material was subjected to successive solvent extraction using petroleum ether, benzene, chloroform, and ethanol in a Soxhlet apparatus. Each extract was concentrated under reduced pressure to remove the solvent and dried in a vacuum desiccator. The aqueous extract was prepared separately by maceration with double-distilled water, followed by concentration on a water bath and drying in a desiccator. The dried extracts were weighed and stored for further analysis.

2.3 Determination of Percentage Yield

The percentage yield of each extract was calculated using the following formula:

Percentage yield (% w/w) = (Weight of dried extract / Weight of powdered crude drug) × 100

2.4 Preliminary Phytochemical Screening

All extracts were subjected to preliminary qualitative phytochemical screening for the detection of the following constituents using standard procedures:

- Carbohydrates
- Alkaloids
- Glycosides
- Tannins
- Steroids
- Triterpenoids
- Volatile oils
- Fixed oils and fats
- Flavonoids
- Polyphenols
- Saponins
- Amino acids
- Gums and mucilage

2.5 Estimation of Total Phenolic Content

The total phenolic content of various extracts was determined by the Folin–Ciocalteu colorimetric method. Quercetin was used as the reference standard. Standard quercetin solutions in the concentration range of 2–10 µg/mL were prepared, and absorbance was measured at 765 nm using a UV-visible spectrophotometer. A calibration curve was plotted, and the total phenolic content of the extracts was calculated and expressed as mg quercetin equivalent (QE)/g of extract.

2.6 HPTLC Fingerprinting of Ethanolic Extract

HPTLC analysis of the ethanolic extract of *Stachytarpheta urticifolia* was carried out using silica gel 60 F254 precoated aluminum plates.

Chromatographic conditions

- **Stationary phase:** Silica gel 60 F254 plates

- **Mobile phase:** Ethyl acetate:formic acid:water (6:1:1)
- **Sample application:** CAMAG Linomat applicator
- **Development chamber:** CAMAG twin-trough chamber
- **Detection reagent:** Anisaldehyde–sulphuric acid reagent
- **Reference standard:** Quercetin

The developed plates were dried, derivatized, and scanned under suitable wavelength conditions to obtain the chromatographic fingerprint profile.

2.7 In Vitro Antioxidant Activity

The ethanolic extract of *Stachytarpheta urticifolia* (EESU) was selected for in vitro antioxidant evaluation based on its higher phenolic content and phytochemical richness. Quercetin was used as the standard antioxidant.

2.7.1 Superoxide Radical Scavenging Assay

Superoxide radical scavenging activity was determined by the PMS–NADH–NBT method. The reaction mixture contained nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), and different concentrations of EESU. Absorbance was measured at 560 nm. The decrease in absorbance indicated scavenging of superoxide radicals.

2.7.2 Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was determined by the deoxyribose degradation method using the Fe^{3+}–ascorbate–EDTA–H_2O_2 system. The reaction mixture was incubated and treated with thiobarbituric acid (TBA) and trichloroacetic acid (TCA). The absorbance of the resulting chromogen was measured at 532 nm.

2.7.3 Lipid Peroxidation Inhibition Assay

Lipid peroxidation inhibitory activity was evaluated using rat liver homogenate as the lipid-rich medium. Lipid peroxidation was induced using Fe^{2+}/ascorbate, and the formation of thiobarbituric acid reactive substances (TBARS) was measured spectrophotometrically at 532 nm after reaction with TBA.

2.8 Calculation of Percentage Inhibition

The percentage inhibition of free radicals or lipid peroxidation was calculated using the following equation:

% Inhibition = [(Acontrol – Atest) / Acontrol] × 100

Where:

- **Acontrol** = Absorbance of control
- **Atest** = Absorbance of test sample

2.9 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis may be performed using one-way ANOVA followed by appropriate post hoc test. A value of $p < 0.05$ may be considered statistically significant.

3. RESULTS

3.1 Preliminary Phytochemical Screening

The phytochemical screening of various extracts of *Stachytarpheta urticifolia* revealed the presence of different classes of secondary metabolites. The ethanolic extract showed the presence of carbohydrates, alkaloids, glycosides, tannins, triterpenoids, flavonoids, polyphenols, saponins, and amino acids, indicating its rich phytochemical composition.

Table 1. Preliminary phytochemical screening of various extracts of *Stachytarpheta urticifolia*

Sl. No.	Phytochemical Test	Petroleum Ether	Benzene	Chloroform	Ethanol	Water
1	Carbohydrates	-	-	-	+	+
2	Alkaloids	-	-	-	+	+
3	Glycosides	-	-	-	+	+
4	Tannins	-	-	-	+	+
5	Steroids	+	+	+	-	-
6	Triterpenoids	+	+	+	+	-
7	Volatile oils	-	-	-	-	-
8	Fixed oils and fats	-	-	-	-	-

Sl. No.	Phytochemical Test	Petroleum Ether	Benzene	Chloroform	Ethanol	Water
9	Flavonoids	-	-	-	+	+
10	Polyphenols	-	-	-	+	+
11	Saponins	-	-	-	+	+
12	Amino acids	-	-	-	+	+
13	Gums and mucilage	-	-	-	-	+

3.2 Percentage Yield and Total Phenolic Content

Among all extracts, the ethanolic extract exhibited the highest percentage yield and total phenolic content, suggesting efficient extraction of phenolic compounds by ethanol.

Table 2. Percentage yield and total phenolic content of various extracts

Extract	Percentage Yield (% w/w)	Total Phenolic Content (mg QE/g extract)
Petroleum ether	5.39	30.21 ± 2.37
Benzene	2.55	21.29 ± 2.17
Chloroform	10.24	19.04 ± 3.41
Ethanol	26.63	254.66 ± 19.55
Water	20.17	182.92 ± 12.16

3.3 HPTLC Fingerprinting

HPTLC fingerprinting of the ethanolic extract revealed multiple peaks indicating the presence of various phytoconstituents. A prominent peak observed at **Rf 0.429** corresponded to the standard quercetin peak, confirming the presence of quercetin in the ethanolic extract.

Table 3. HPTLC peak profile of ethanolic extract of *Stachytarpheta urticifolia*

Peak	Start Rf	Max Rf	End Rf	Max Height	Area	Area %
1	0.109	0.177	0.197	98.6	3868.2	6.01
2	0.303	0.318	0.318	118.5	4186.3	6.42
3	0.318	0.352	0.398	159.7	5135.5	7.62
4	0.398	0.429	0.442	242.9	10625.6	10.73
5	0.442	0.488	0.487	323.6	10247.9	12.23
6	0.487	0.494	0.504	258.8	7462.7	10.65
7	0.504	0.518	0.548	288.8	13253.5	18.11
8	0.548	0.566	0.578	173.9	5317.6	5.88
9	0.578	0.591	0.629	193.4	9637.2	11.44
10	0.629	0.642	0.656	121.3	4269.4	6.51
11	0.656	0.699	0.714	99.9	3417.3	4.42

Quercetin content in ethanolic extract: approximately **0.214 mg/g of extract**

3.4 In Vitro Antioxidant Activity

3.4.1 Superoxide Radical Scavenging Activity

The ethanolic extract exhibited concentration-dependent superoxide radical scavenging activity and showed better inhibition than the standard at all tested concentrations.

Table 4. Superoxide radical scavenging activity of EESU

Concentration (µg/mL)	% Inhibition by EESU	% Inhibition by Quercetin
10	39.123 ± 5.152	28.342 ± 2.789
20	55.239 ± 5.476	44.438 ± 3.234
30	67.457 ± 4.421	63.293 ± 2.536
40	77.921 ± 3.210	74.134 ± 2.672
50	89.111 ± 3.145	87.846 ± 3.436

3.4.2 Hydroxyl Radical Scavenging Activity

The ethanolic extract demonstrated significant hydroxyl radical scavenging activity in a concentration-dependent manner.

Table 5. Hydroxyl radical scavenging activity of EESU

Concentration (µg/mL)	% Inhibition by EESU	% Inhibition by Quercetin
10	44.255 ± 4.825	29.618 ± 2.364
20	56.324 ± 4.053	44.302 ± 1.532
30	64.298 ± 2.496	62.322 ± 3.162
40	75.573 ± 3.083	75.635 ± 3.127
50	89.262 ± 2.166	89.428 ± 2.364

3.4.3 Lipid Peroxidation Inhibition Activity

The ethanolic extract exhibited strong inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation in rat liver homogenate.

Table 6. Lipid peroxidation inhibition activity of EESU

Concentration (µg/mL)	% Inhibition by EESU	% Inhibition by Quercetin
50	33.135 ± 2.615	23.422 ± 3.126
100	45.814 ± 3.513	38.868 ± 3.323
150	58.394 ± 3.322	49.615 ± 3.742
200	76.702 ± 2.542	64.265 ± 3.603
250	94.686 ± 3.124	88.844 ± 3.128

4. DISCUSSION

The present study demonstrated that the ethanolic leaf extract of *Stachytarpheta urticifolia* possesses significant in vitro antioxidant activity, which may be attributed to its rich phytochemical composition. Preliminary phytochemical screening confirmed the presence of flavonoids, polyphenols, tannins, alkaloids, saponins, and triterpenoids in the ethanolic extract. These phytoconstituents are known to contribute to antioxidant activity by scavenging free radicals, donating hydrogen atoms or electrons, and preventing oxidative chain reactions.

Among all extracts, the ethanolic extract showed the highest percentage yield and the highest total phenolic content, indicating that ethanol was the most suitable solvent for extracting phenolic constituents from the plant material. The high phenolic content strongly correlates with the antioxidant activity observed in the present study, as phenolic compounds are known to stabilize free radicals and inhibit oxidative damage.

HPTLC fingerprinting of the ethanolic extract confirmed the presence of quercetin, a well-known flavonoid with potent antioxidant activity. The presence of quercetin in the extract provides a useful chemical marker for standardization and quality control. In addition, the HPTLC chromatogram showed multiple peaks, suggesting that the antioxidant activity of the extract may result from the synergistic effect of several phytoconstituents rather than a single compound alone.

In the superoxide radical scavenging assay, the ethanolic extract showed strong and concentration-dependent scavenging activity. Since superoxide radicals are precursors to more reactive oxygen species, their neutralization is important in preventing oxidative damage. Similarly, the extract exhibited significant hydroxyl radical scavenging activity, which is especially relevant because hydroxyl radicals are highly reactive and capable of causing extensive biomolecular damage. The extract also showed pronounced inhibition of lipid peroxidation in rat liver homogenate, indicating its potential to protect biological membranes against oxidative degradation.

Overall, the results support the traditional medicinal use of *Stachytarpheta urticifolia* and suggest that its ethanolic leaf extract may serve as a valuable natural source of antioxidant phytoconstituents. However, further studies including isolation of active compounds, determination of IC50 values, in vivo antioxidant evaluation, and toxicological profiling are necessary to confirm its therapeutic applicability.

5. CONCLUSION

The present study revealed that the ethanolic leaf extract of *Stachytarpheta urticifolia* possesses significant in vitro antioxidant activity, as evidenced by its superoxide radical scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibitory effects. The extract was found to contain a wide range of phytoconstituents, particularly flavonoids and phenolic compounds, which are likely responsible for the observed activity. The high total phenolic content and the confirmation of quercetin by HPTLC further support the antioxidant potential of the extract.

These findings scientifically validate the traditional use of *Stachytarpheta urticifolia* and indicate that the plant may serve as a promising source of natural antioxidant agents. Further studies are recommended to isolate and characterize the active constituents and to establish the efficacy and safety of the extract through in vivo pharmacological investigations.

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