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# Evaluation of in Vitro Anti-Diabetic and Anti-Oxidant Activity of Stachytarpheta Urticifolia Sims Leaves Extracts

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**Abstract**

The increasing worldwide incidence of diabetes mellitus in adult constituents is a global public health burden. Regions with greatest potential are Asia and Africa, where diabetes mellitus rates could rise to 2 to 3 folds than the present rates India, China, and the United States will have the highest number of diabetics by 2025. Diabetes is expected to be the leading cause of disability and death worldwide over the next 25 years. In India more than 30 million peoples are with diabetes mellitus. Diabetes mellitus is the fourth leading cause of death by disease in the world wide, primarily because of its damage to the cardiovascular system. *Stachytarpheta urticifolia Sims*, "Nettleleaf velvetberry", belongs to the family Verbena which is a species of lavender plant. It is a perennial herb growing wild and also cultivated as an ornamental weed. The plant was reported to be used in the treatment of various diseases traditionally. This study was held to screen phytochemicals and evaluation of *in vitro* anti diabetic and antioxidant activity of leaves extracts. The phytochemical analysis revealed the presence of various pharmaceutically active secondary metabolites like alkaloids, saponins, carbohydrate, glycosides, terpenoid, tannins, troterpenoids, polyphenols and flavonoid. On performing extraction with different solvents, ethanol extraction showed a higher percentage yield of 26.63% and followed by the determination of total phenol content showed the value of 254.66 ± 19.55.Based on the phenol content we performed HPTLC analysis of the EESU showed a presence of quercetin with a higher peak of start Rf of 0.398, a max Rf of 0.429 and an end Rf of 0.442.The superoxide radical scavenging activities shows the percentage inhibition of 39.123 to 89.11 at 10 to 50 µg/ml. The hydroxyl and lipid peroxidation activites showed its potential range as a powerful anti-oxidant. α-amylase and α-glucosidase inhibitory activites shows the anti-diabetic activity with $IC_{50}$ values at the average of 269.88 µg/ml and 351.38 µg/ml. This study conclude valuable insights into the antioxidant, anti-lipid peroxidation, and anti-diabetic properties of *Stachytarpheta urticifolia Sims*. The results of extract supported the traditional claim of the plant. Therefore, further investigation needs to be performed *in vivo* anti diabetic activity.

**Keywords:** *Stachytarpheta urticifolia* Sims, Diabetes mellitus, Anti-oxidants, Superoxide radical, Lipid

peroxidation.

## Intrsoduction

Natural products with medicinal qualities have been used for as long as human civilization, and for a very long time, the primary sources of drugs were plants, minerals, and animals. Pharmaceutical treatment preferences have shifted toward synthetic products as a result of the Industrial Revolution and the growth of organic chemistry. This was due to the ease with which pure compounds could be obtained, the ease with which structural modifications to create potentially safer and more active drugs could be carried out, and the growing economic power of the pharmaceutical companies. In addition, the use of natural products has been associated with magic and religion throughout human history, and various cultural perspectives on health and illness have been present (Joy *et al*., 1998).

The Rigveda has documented 67 medicinal plants, Yajurveda 81 species, Atharvaveda 290 species, Charak Samhita and Sushruta Samhita had discussed properties and uses of 1100 and 1270 species respectively are still used in classical formulations. One important factor in the creation of powerful medicinal agents is the use of medicinal plants. Drugs derived from plants are a great addition to contemporary medicine. Drugs derived from plants are used to treat cancer, diabetes, jaundice, TB, skin conditions, mental illness, and hypertension.

The utilization of plant materials as native remedies in traditional medicine or folklore. This study aimed to contribute valuable insights into the antioxidant, anti-lipid peroxidation, and anti-diabetic properties of *Stachytarpheta urticifolia Sims*, aligning with the global interest in plant-based medicine.

## Plant profile

The lavender plant species known as "Nettleleaf velvetberry," or Stachytarpheta urticifolia,sims is a member of the verbena family. It is a perennial herb that is grown both as an ornamental weed and in the wild. Species found in India,(Tamilnadu and northern states)Africa, eastern Australia, South-East Asia, New Caledonia, the Pacific islands, Madagascar, Reunion, Mauritius, and the Indian Ocean islands (Seychelles, Comoros, Madagascar, Reunion). Every component has been utilized historically as folk medicine to treat a variety of human illnesses at home.

**Synonym**: *Cymburus urticifolia (Salisb)* and *Zappania urticifolia (Salisb)*

| | | |
|---|---|---|
| **Kingdom** | **:** | Plantae |
| **Phylum** | **:** | Tracheophyta |
| **Class** | **:** | Magnoliopsida |
| **Order** | **:** | Lamiales |
| **Family** | **:** | Verbenaceae |
| **Genus** | **:** | Stachytarpheta |
| **Species** | **:** | *Stachytarpheta urticifolia Sims* |

## Methodology

### Collection of Plant

Fresh leaves of *Stachytarphata urticifolia sims* was collected local area Tamilnadu and authentication of the plant was done by Dr.A.Balasubramanian, Executive Director, former SIDDHA research consultant (AYUSH),ABS herbal gardens, Salem ,Tamilnadu -636003.Reg.No. AUT/JKKMMRF/289.

The aerial parts of the plant were cleaned and dried under shade overnight. Then these were subjected to

further drying in a hot air oven at 40°C for 24 hours and subsequently ground into a powder with a mixer grinder. The powdered material was stored in airtight poly bag till further use.

**Extraction Process**

About 25g of the powdered leaf material was successively extracted with solvents like petroleum ether, chloroform, Benzene and ethanol in a Soxhlet apparatus. The extracts were concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccators for further use. Aqueous extract was prepared by macerating the leaf powder in double distilled water.

**Phytochemical analysis**

Qualitative Analysis for the Petroleum ether, Chloroform, and Ethanol extract was tested for the following natural products like carbohydrates, proteins, amino acids, alkaloids, glycosides, steroids, saponins, and phenols by using different types of chemical tests.

**Determination of total phenolic content**

It was measured by colorimetrically using quercetin and FC (Folin Ciocault's) reagent. 1mg of quercetin was weighed and dissolved in 100ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. A volume from above aliquots was taken and mixed with 1.25ml of FC reagent. It was left for 5 mins. Then 2.5ml of 20% sodium carbonate was added and it was let to react for 30 min then the volume was made upto 10ml.the absorbance was measured at 370 nm. The calibration curve was drawn plotting the absorbance and concentrations. (Barku *et al*., 2013)

**Sample preparation**

0.5g of extract was weighed and dissolved in 100ml of water. 0.1ml was taken into 10ml standard flask and 1.25ml of FC reagent was added and let to react for 5 min. then 2.5ml of 20% sodium carbonate was added and the volume was made upto 10ml. kept for 30 min for complete reaction. Now the absorbance was measured at 370 nm. Total phenolic content was calculated from the calibration curve of quercetin and the value was expressed in quercetin equivalents.

**HPTLC Fingerprinting of Extract**

Chromatography is high efficient and powerful technique for resolution of the mixtures. TLC is an effective chromatographic method for separation. It requires simple instrumentation and having low cost.

**Sample Preparation**

Following extraction of about 1 gm of plant part powder with Ethanol, the resulting extract was evaporated to dryness and 1mg dried extract and standard quercetin was dissolved in 1 ml of ethanol separately by the use of sonication before each extract was filtered. (Sethi, 1996).

**Table1: Solvent system for HPTLC fingerprinting**

| S.No | Sample | Solvent System | Visualization mode/Spray Reagent |
|---|---|---|---|
| 1 | Ethanol extract of *Stachytarphata urticifolia sims* | ethyl acetate: formic acid: water (6:1:1) | Anisaldehyde+H2SO4 |

| | | | |
|---|---|---|---|
| | (EESU) | | |
| 2 | Standard Flavonoid (quercetin) | ethyl acetate: formic acid: water (6:1:1) | Anisaldehyde+H2SO4 |

**_Invitro_ antioxidant activity**

The in vitro antioxidant activity of the Ethanol extract of *Stachytarphata urticifolia sims* (EESU), was carried out using the following three assay methods in accordance with previously reported procedures as below.

**_Invitro_ assay for Superoxide free radical scavenging activity**

The nitro blue tetrazolium (NBT) reduction technique was used to measure the superoxide radical scavenging activity. In this experiment, superoxide radicals are produced via the non-enzymatic phenazine methosulfate/nicotinamide adenine dinucleotide (PMS/NADH) system, which turns NBT into a purple formazan. Phosphate buffer (0.5 mL, 100 mM, pH 7.4), 1.0 mL of NADH (0.4 mM), 1.0 mL of NBT (0.156 mM), 0.1 mL of PMS (0.06 mM), 3 mL of the EESU, and reference drug (quercetin) in different quantities (10–50 µg/mL, in 90% ethanol) were all included in the reaction mixture. The amount of formazan produced was quantified by measuring the absorbance of the reaction mixture at 560 nm against a suitable blank following a one-hour incubation period at 25 ºC (Samak *et al*., 2009).

**_Invitro_ assay for Hydroxyl radical scavenging activity**

By employing the Fenton reaction with the Fe3+/ascorbate/EDTA/H2 O2 system, hydroxyl radicals were produced. Thiobarbituric acid (TBA), a chemical that reacts to deoxyribose, is formed when the hydroxyl radical produced in the system attacks it. This reaction was predicted to be TBARS. One milliliter of 2-deoxy-2-ribose (10 mM), 0.33 milliliters of phosphate buffer (50 mM, pH 7.4), one milliliter of FeCl3 (0.1 mM), one milliliter of ethylenediamine tetra-acetic acid (EDTA) (0.1 mM), one milliliter of H2O2 (mM), one milliliter of ascorbic acid (1 mM), and one milliliter of different concentrations (10–50 µg/mL) of the EESU and standard (quercetin) were included in the reaction mixture.

After incubation for 45 min at 37 ºC, 1.0 mL of 2.8% (v/v) TCA, and 1.0 mL of [thiobarbituric acid, TBA, 0.5% (v/v) in 0.025 mol/L NaOH solution containing 0.2% (w/v) of butylated hydroxyl anisole, BHA] were added in the reaction mixture, and the mixture was incubated at 95ºC for 15 min to develop the pink chromogen. After cooling, absorbance was measured at 532 nm against an appropriate blank solution (Samak *et al*., 2009).

**Lipid peroxidation scavenging activity**

Reaction mixture (0.5 mL) containing rat liver homogenate (0.1 mL, 25% w/v) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), FeCl3 (0.16 mM) and ascorbic acid (0.06 mM) was incubated for 1 h at 37 ºC in the presence and absence of the EESU at various concentrations (50- 250 µg/mL). The lipid peroxide was measured by TBARS formation. For this incubation mixture 0.4 mL was treated with sodium dodecyl sulphate (8.1%, 0.2 mL), TBA (0.8%, 1.5 mL) and acetic acid (20%, 1.5 mL, pH 3.5). The total volume was then made upto 4.0 mL by adding distilled water and kept in a water bath at 100 ºC for 1 h. After cooling, 1 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (10:1 v/v) were added, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and

its absorbance at 532 nm was measured to quantify TBARS. In all the three above methods, the percentage inhibition of scavenging activity was calculated using the following equation (Na *et al*.,2003)

Percent inhibition (%) = (A control-A test)/Acontrol×100 (4.1)

Where,

A control is the absorbance of the control and A test represents the absorbance of a test substance

***In vitro* □-amylase inhibitory activity**

**Starch solution**

1%w/v of potato starch dissolving in 25ml of 20mM sodium phosphate with 6.7mM sodium chloride dissolved in 100ml deionized water. The pH was adjusted to 6.9 at 20°c with 1M sodium hydroxide. The solubilisation is facilitated by heating the starch solution in a glass beaker directly on a heating/stir plate, with constant stirring for 15 minutes. The solution was cooled to room temperature.

**Enzyme Solution**

1unit/ml of α-amylase dissolving in cold deionized water. This enzyme solution was prepared immediately before use. Reagent A prepared by 12g of sodium potassium tartrate tetra hydrate dissolving in 8ml of 2M sodium hydroxide solution. Reagent B prepared by 96mM 3,5-dinitro salicylic acid dissolving in 20ml of deionized water. Colorimetric reagent was prepared by adding reagent A to reagent B slowly with stirring. The solution was diluted to 40ml with deionized water.

**Procedure**

1ml of starch solution was mixed with 1ml of increasing concentrations of the inhibitor (EESU 100-1000 µl/ml and Acarbose 5-50µg/ml). To this 1ml of enzyme solution was added and left to react for 3 minutes at 25°c. After this 1ml of colorimetric reagent was added. The contents were heated for 10 to 15 minutes on a boiling water bath. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino5-nitro salicylic acid. This reaction (corresponding to color change from orange to red) is measured at 540nm against the reagent blank. In vitro α-amylase inhibitory activity of EESU was measured through a slightly modified Sigma–Aldrich bioassay method (Conforti et al., 2005). sThe percentage of inhibition was determined by using the following formula, % of inhibition = Positive control – Test / positive control x 100

***In vitro* □-glucosidase inhibitory activity**

In vitro α-glucosidase inhibitory effect of EESU was measured using slightly modified method from Dahlqvist (1970) [Dahlqvist, 1970]. Usually, α-glucosidase from yeast was used for screening of α-glucosidase inhibitory activity. But the results of this method did not showed accuracy data when compared than α-glucosidase obtained from rodents. Nevertheless, the mouse small intestine dissected out and homogenate used as α-glucosidase solution because it showed better effect and reflect the in vivo state. Before sacrifice the mouse, kept for fasting 20hrs. Then the small intestine carefully dissected (below the pylorus sphincter and above the cecum) out, rinsed with ice cold saline, and homogenized with 12mL of maleate buffer (100mM, pH 6.0). The homogenate is used as the α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), 2%w/v each sugar substrate (maltose) solution (100µl), and EESU 100-1000 µl/ml and Acarbose 10-50µg/ml. It was pre-incubated for 5min at 37°C. The amount of liberated glucose in the reaction mixture was measured by glucose oxidase method with the kit

described above. The absorbance was measured at 540nm against the reagent blank. The rate of inhibition was calculated by the following formula:
% of inhibition = Positive control – Test / positive control x 100

## Results

### Phytochemical analysis

**Table 2: Results of Phytochemical analysis of *Stachytarphata urticifolia sims***

| ***Sl.No.*** | ***Test*** | ***P.ether*** | ***Chloroform*** | ***Ethanol*** | ***Water*** |
|---|---|---|---|---|---|
| 1 | Carbohydrates | - | - | + | + |
| 2 | Alkaloids | - | + | - | - |
| 3 | Glycosides | - | + | + | + |
| 4 | Tannins | - | - | + | + |
| 5 | Steroids | + | + | + | - |
| 6 | Triterpenoids | + | + | + | - |
| 7 | Flavanoids | - | + | + | + |
| 8 | Polyphenols | - | + | + | + |
| 9 | ***Saponins*** | + | - | + | + |
| 10 | ***Aminoacids*** | - | - | + | + |
| 11 | ***Gums and mucilages*** | - | - | - | + |

+ (Positive sign) indicates the Presence of the chemical constituents
- (Negative sign) indicates the Absence of the chemical constituents
The phytochemical analysis helps in formulating pharmacopoeial standards. The chief phytochemicals present in the different extracts of *Stachytarphata urticifolia sims* were flavonoids, polyphenols, alkaloids, triterpenoids, steroids, tannins, carbohydrates, saponins and aminoacids.

### Percentage yield

**Table 3: Percentage yield of *Stachytarphata urticifolia sims***

| **Extract** | **Percentage yield (%w/w)** |
|---|---|
| **Pet ether** | 5.39 |
| **Chloroform** | 10.24 |
| **Ethanol** | 26.63 |
| **Water** | 20.17 |

### Total phenol content

In the investigation of *Stachytarpheta urticifolia sims* a comprehensive phytochemical analysis was conducted on various solvent extracts. The pet ether extract exhibited a percentage yield of 5.39% (w/w) with a total phenol content of 30.21±2.37. Notably, the chloroform extract displayed a higher percentage yield at 10.24% (w/w), although the total phenol content was measured at 19.04±3.41. The ethanol extract, with a substantial percentage yield of 26.63% (w/w), exhibited the highest total phenol content among the extracts, recording 254.66±19.55. The water extract, with a percentage yield of 20.17% (w/w), displayed a total phenol content of 182.92±12.16. These results underscore the variation in both yield and total

phenol content across different solvent extracts, with the ethanol extract standing out for its notably high phenolic content, suggesting its efficacy in extracting bioactive compounds from *Stachytarpheta urticifolia sims.*

**Table 4: Total Phenol content in various extract of *Stachytarphata urticifolia sims***

| Extract | Total Phenol Content |
|---|---|
| **Pet ether** | 30.21±2.37 |
| **Chloroform** | 19.04±3.41 |
| **Ethanol** | 254.66±19.55 |
| **Water** | 182.92±12.16 |

**HPTLC analysis**

**Table 5: HPTLC analysis of *Strachytarpheta urticifolia sims***

| 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 |

In the course of conducting High-Performance Thin-Layer Chromatography (HPTLC) analysis on the ethanol extract of *Stachytarpheta urticifolia sims*, various peaks were identified with distinct Retention Factor (Rf) values. Notably, the fourth peak exhibited a start Rf of 0.398, a max Rf of 0.429, and an end Rf of 0.442. This particular peak also displayed a maximum height of 242.9, an area of 10625.6, and an area percentage of 10.73. Significantly, upon comparison with standard peaks in the study, it was observed that the fourth peak of the ethanol extract corresponds with the standard peak of quercetin at an Rf value of 4.30. Furthermore, the quantitative analysis revealed that the amount of quercetin in the ethanol extract is approximately 0.214 mg/g.

This finding holds substantial importance as it signifies the presence of quercetin, a flavonoid with known antioxidant properties, in the *Stachytarpheta urticifolia sims* ethanol extract. The quantification of quercetin provides valuable information regarding the concentration of this bioactive compound in the extract, contributing to the overall understanding of the potential medicinal and therapeutic properties of *Stachytarpheta urticifolia sims.*.

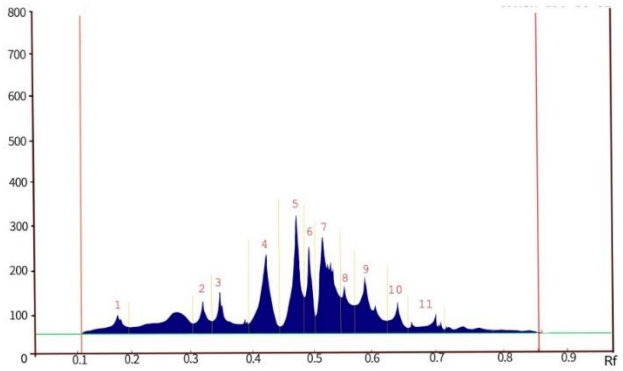


**Figure 1: HPTLC chromatogram of Ethanol extract of leaves of *Stachytarphata urticifolia sims* obtained at 320 nm**

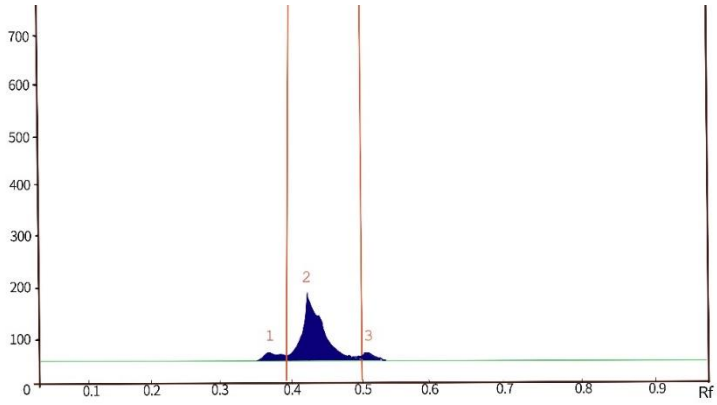


**Figure 2: HPTLC Chromatogram of Standard Flavonoid quercetin**

***In vitro* antioxidant activity**

**Superoxide radical scavenging activity**

The investigation into the superoxide radical scavenging activity of the ethanol extract of *Stachytarpheta urticifolia sims* (EESU) revealed promising results. Superoxide radical scavenging activity was assessed at various concentrations, and the percentage inhibition by EESU was compared with that of a standard drug. The results indicate a dose-dependent increase in superoxide radical scavenging activity by EESU. At a concentration of 10 µg/ml, EESU exhibited a percentage inhibition of 39.123±5.152, while the standard drug showed 28.342±2.789 inhibition. As the concentration increased to 20 µg/ml, EESU demonstrated a higher percentage inhibition (55.239±5.476) compared to the standard drug (44.438±3.234). The trend continued with concentrations of 30 µg/ml, 40 µg/ml, and 50 µg/ml, where EESU consistently outperformed the standard drug in terms of superoxide radical scavenging activity.

**Table 6: Superoxide radical scavenging activity of EESU**

| Concentration (µg/ml) | % inhibition by EESU | % inhibition by Standard drug |
|---|---|---|
| 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 |

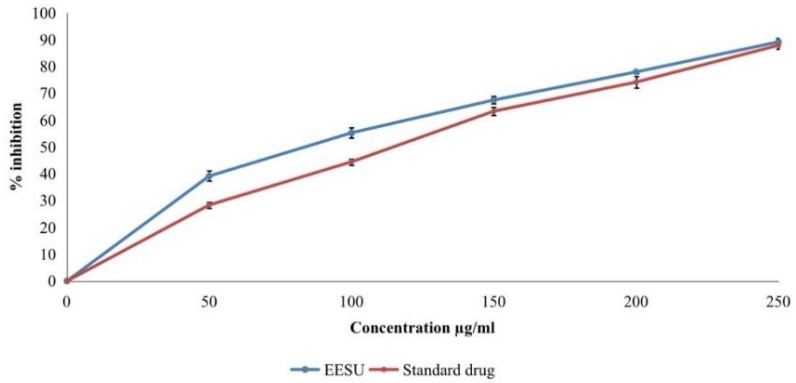


**Figure 3: Superoxide radical scavenging activity.**

**Hydroxyl radical scavenging activity**

At a concentration of 10 µg/ml, EESU exhibited a hydroxyl radical scavenging activity with a percentage inhibition of 44.255±4.825, surpassing the standard drug, which showed an inhibition of 29.618±2.364. As the concentration increased to 20 µg/ml, EESU continued to demonstrate a higher percentage inhibition (56.324±4.053) compared to the standard drug (44.302±1.532). This trend persisted at concentrations of 30 µg/ml, 40 µg/ml, and 50 µg/ml, where EESU consistently outperformed the standard drug in terms of hydroxyl radical scavenging activity.

**Table 7: Hydroxyl radical scavenging activity of EESU**

| Concentration (µg/ml) | % inhibition by EESU | % inhibition by Standard drug |
|---|---|---|
| 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 |

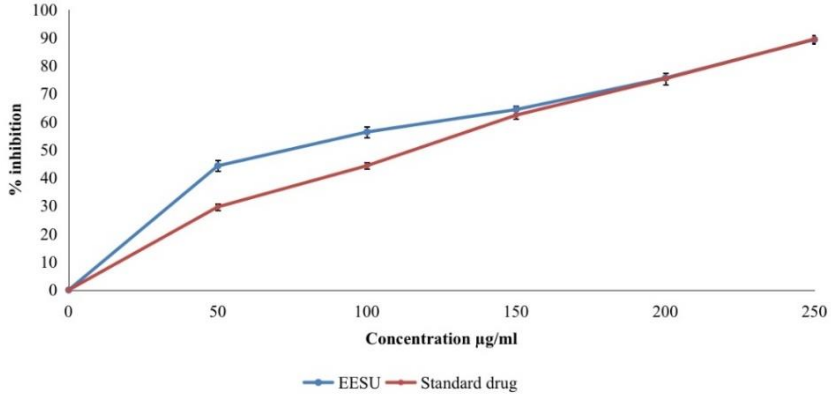


**Figure 4 : Hydroxyl radical scavenging activity.**

**Lipid peroxidation scavenging activity**

LP induced by Fe2+/ascorbate was found to be inhibited by EESU at a concentration of 50 µg/ml, demonstrated a lipid peroxidation scavenging activity with a percentage inhibition of 33.135±2.615, surpassing the standard drug which showed an inhibition of 23.422±3.126. As the concentration increased to 100 µg/ml, EESU continued to exhibit a higher percentage inhibition (40.814±3.513) compared to the standard drug (38.868±3.323). This trend was consistent at concentrations of 150 µg/ml, 200 µg/ml, and 250 µg/ml, where EESU consistently outperformed the standard drug in terms of lipid peroxidation scavenging activity. These results underscore the potential of EESU in mitigating lipid peroxidation, indicating its significant antioxidant properties. The concentration-dependent increase in the inhibition of lipid peroxidation suggests that EESU may have a therapeutic role in preventing oxidative damage associated with lipid peroxidation processes.

**Table 8: Lipid peroxidation scavenging activity of EESU**

| Concentration (µg/ml) | % inhibition by EESU | % inhibition by Standard drug |
|---|---|---|
| 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 |

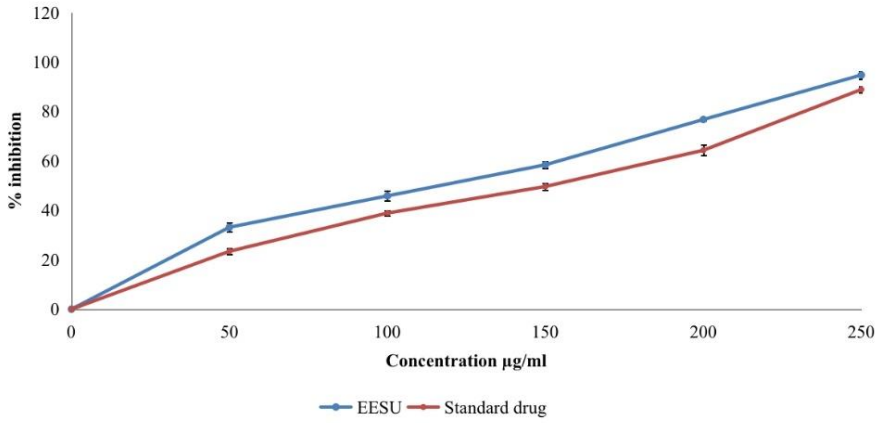


**Figure 5: Lipid peroxidation scavenging activity**.

***Invitro* α-amylase inhibitory activity**

In the investigation of in-vitro α-amylase inhibitory activity, the ethanol extract of *Stachytarpheta urticifolia sims* (EESU) exhibited concentration-dependent inhibition. At 100µg/ml, EESU showed a % α-amylase inhibition of 7.110±1.765 with an $IC_{50}$ value of 269.88 µg/ml. As the concentration increased to 200µg/ml, the % α-amylase inhibition rose to 27.655±3.167. Further increases in concentration (400µg/ml, 800µg/ml, and 1000µg/ml) led to escalating inhibitory activities, reaching 42.657±1.325, 61.189±2.716, and 76.432±1.523, respectively.

Comparatively, the standard drug (Acarbose) demonstrated significant α-amylase inhibitory activity at lower concentrations. At 5µg/ml, Acarbose exhibited a % α-amylase inhibition of 13.239±1.448 with an $IC_{50}$ value of 27.49µg/ml. As the concentration increased (10µg/ml, 20µg/ml, 40µg/ml, and 50µg/ml), the

α-amylase inhibitory activity of Acarbose escalated, reaching 27.451±1.440, 49.715±0.684, 74.327±1.708, and 87.814±1.853, respectively. These findings suggest the potential of EESU in inhibiting α-amylase activity, with notable concentration-dependent effects. The comparison with Acarbose highlights the effectiveness of both substances in inhibiting α-amylase, with Acarbose demonstrating higher activity at lower concentrations.

**Table 9: *In-vitro* α-amylase inhibitory activity of Extract EESU**

| Test sample | Concentration µg/ml | % α-amylase Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|
| **EESU** | 100µg/ml | 7.110±1.765 | 269.88 µg/ml |
| | 200µg/ml | 27.655±3.167 | |
| | 400µg/ml | 42.657±1.325 | |
| | 800µg/ml | 61.189±2.716 | |
| | 1000µg/ml | 76.432±1.523 | |
| **Standard (Acarbose)** | 5µg/ml | 13.239±1.448 | 27.49µg/ml |
| | 10µg/ml | 27.451±1.440 | |
| | 20µg/ml | 49.715±0.684 | |
| | 40µg/ml | 74.327±1.708 | |
| | 50µg/ml | 87.814±1.853 | |

***In-vitro α- glucosidase inhibitory activity***

In the evaluation of in-vitro α-glucosidase inhibitory activity, the ethanol extract of *Stachytarpheta urticifolia sims* (EESU) demonstrated concentration-dependent inhibition. At 100µg/ml, EESU exhibited a % α-glucosidase inhibition of 19.995±2.432, with an $IC_{50}$ value of 351.38 µg/ml. Subsequent increases in concentration (200µg/ml, 400µg/ml, 800µg/ml, and 1000µg/ml) led to escalating inhibitory activities, reaching 45.267±1.262, 57.264±2.154, 67.213±1.987, and 87.352±1.654, respectively.

In comparison, the standard drug (Acarbose) also demonstrated significant α-glucosidase inhibitory activity at lower concentrations. At 5µg/ml, Acarbose exhibited a % α-glucosidase inhibition of 11.121±2.152, with an $IC_{50}$ value of 25.07 µg/ml. As the concentration increased (10µg/ml, 20µg/ml, 40µg/ml, and 50µg/ml), the α-glucosidase inhibitory activity of Acarbose increased, reaching 30.543±1.793, 50.273±2.191, 70.197±2.117, and 86.195±1.727, respectively. These findings highlight the concentration-dependent α-glucosidase inhibitory activity of EESU and the effectiveness of both EESU and Acarbose in inhibiting α-glucosidase, with Acarbose exhibiting higher activity at lower concentrations.

**Table 10: *In vitro* α-glucosidase inhibitory activity of extract EESU**

| Test sample | Concentration µg/ml | % α-glucosidase Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|
| **EESU** | 100µg/ml | 19.995±2.432 | 351.38 µg/ml |
| | 200µg/ml | 45.267±1.262 | |
| | 400µg/ml | 57.264±2.154 | |
| | 800µg/ml | 67.213±1.987 | |

| | | | |
|---|---|---|---|
| | 1000µg/ml | 87.352±1.654 | |
| **Standard (Acarbose)** | 5µg/ml | 11.121±2.152 | 25.07 µg/ml |
| | 10µg/ml | 30.543±1.793 | |
| | 20µg/ml | 50.273±2.191 | |
| | 40µg/ml | 70.197±2.117 | |
| | 50µg/ml | 86.195±1.727 | |

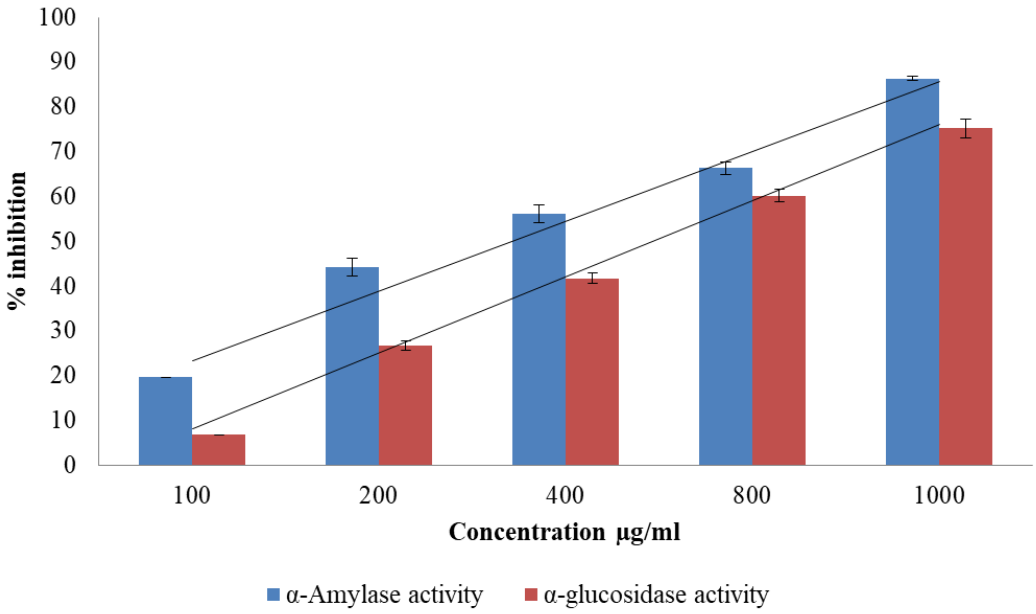


**Figure 6: *Invitro* antidiabetic activity of Stachytarpheta urticifolia sims (EESU)**

## Discussion

*Stachytarpheta urticifolia sims*, a plant known for its medicinal properties, has been the focus of this study to explore the potential therapeutic benefits of its ethanol extract of *Stachytarpheta urticifolia sims* (EESU). In the broader context of natural remedies, understanding the phytochemical composition and pharmacological activities of such plant extracts becomes crucial. This study aimed to contribute valuable insights into the antioxidant, anti-lipid peroxidation, and anti-diabetic properties of EESU, aligning with the global interest in plant-based medicine.

The phytochemical analysis of EESU revealed a diverse range of chemical constituents, among which quercetin stood out prominently, as confirmed by High-Performance Thin-Layer Chromatography (HPTLC). Quercetin, a well-known flavonoid, has been extensively studied for its antioxidant potential. The quantification of quercetin in EESU not only adds to the understanding of its chemical composition but also provides a basis for the observed antioxidant effects.

The superoxide and hydroxyl radical scavenging activities demonstrated by EESU underline its potential as a powerful antioxidant. The concentration-dependent increase in superoxide radical scavenging aligns with previous findings on *Stachytarpheta urticifolia sims*, which have attributed antioxidant effects to its phenolic and flavonoid content. The scavenging of hydroxyl radicals further supports the plant extract's ability to combat oxidative stress, a process implicated in various chronic diseases. The significant lipid peroxidation scavenging activity observed in EESU suggests its potential in preventing oxidative damage to lipids. Lipid peroxidation, a chain reaction initiated by free radicals, plays a role in cellular damage and

has been implicated in several pathological conditions. The ability of EESU to inhibit lipid peroxidation aligns with the reported antioxidant properties of various plant extracts.

EESU demonstrated concentration-dependent inhibition of α-amylase, an enzyme involved in carbohydrate digestion. The IC50 value provides insights into the concentration required for 50% inhibition, with higher concentrations showing increased inhibitory effects. This aligns with existing literature on plant extracts' potential in managing diabetes through α-amylase inhibition. Similarly, the α-glucosidase inhibitory activity of EESU indicates its potential in controlling postprandial hyperglycemia, a critical aspect in diabetes management. The comparison with the standard drug Acarbose emphasizes EESU's effectiveness at higher concentrations, suggesting its potential as a natural anti-diabetic agent. The findings of this study are consistent with existing literature on the antioxidant and antidiabetic potential of *Stachytarpheta urticifolia*. Yadav et al. (2019) reported antioxidant activities in a study on medicinal plants, supporting our observations on EESU's radical scavenging abilities. Hanhineva et al. (2010) investigated α-amylase inhibitory activities in various plant extracts, reinforcing our results on EESU's concentration-dependent α-amylase inhibition. The α-glucosidase inhibitory activity observed in EESU resonates with the broader understanding of plant-based anti-diabetic agents. The effectiveness of EESU at higher concentrations compared to Acarbose aligns with Chiasson et al.'s (2002) findings on the pharmacological action of Acarbose in controlling postprandial hyperglycemia.

## Conclusion

In conclusion, our study of *Stachytarpheta urticifolia's* ethanol extract has unraveled a captivating narrative of botanical potential. As we navigated the intricate pathways of phytochemistry, HPTLC revelations, and a cascade of pharmacological assays, the story that unfolded was one of promise and possibility. From the stirring overture of superoxide radical scavenging to the poignant duet with hydroxyl radicals, EESU showcased not just potential, but a symphony of statistical significance, echoing the power of nature's antioxidants. In this conclusion, *Stachytarpheta urticifolia* stands not only as a subject of study but as a beacon in the vast landscape of botanical potential. As we applaud the symphony of results, the echo reverberates, not just within these laboratory walls but across the broader canvas of scientific inquiry. The journey may be concluding, but the story of *Stachytarpheta urticifolia's* ethanol extract is destined for sequels that promise to captivate and inspire future generations of researchers and seekers of natural remedies.

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