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RESEARCH ARTICLE

Evaluation of *in vitro* anti-inflammatory and antioxidant activities of *Simarouba glauca* leaf extract

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Abstract

Simarouba glauca is a medicinal plant native to India and other tropical regions of South and Central America. Although this plant is widely used to cure many diseases, a systematic study is lacking to understand its medicinal value. Thus, we focused on analysing the phytochemical composition of *S. glauca* leaf extracts and evaluating *in vitro* anti-inflammatory and antioxidant activities using standard bioassays. The anti-inflammatory activity was conducted using protein denaturation assay, heat-induced hemolysis and lipoxygenase inhibitory assays, which revealed IC_{50} values of 328 µg/mL, 342.6 µg/mL and 432.1 µg/mL, for the aqueous, ethanol and ethyl acetate extracts, respectively. In the hemolysis assay, the aqueous extract provided the highest protection (38.95 ± 0.06%) with an IC50 of 801 µg/mL. Additionally, in the lipoxygenase inhibition assay, the aqueous extract exhibited the highest inhibition (80.3 ± 0.34%) with an IC50 of 40 µg/mL. For antioxidant activity, the aqueous, ethanol and ethyl acetate extracts demonstrated scavenging activity against DPPH and ABTS radicals, with IC_{50} values of 180.7 µg/mL, 209.7 µg/mL and 678.2 µg/mL for DPPH and 75.3 µg/mL, 83.9 µg/mL and 225.8 µg/mL for ABTS, respectively. Further, the FRAP assay showed scavenging activity of 72.28 ± 0.76 µg/28 mL, 72.5±0.75µg/mL and 16.8±0.58 µg/mL and the phosphomolybdate method exhibited significant antioxidant activity of 80.0 ± 0.87 µg/mL, 81.39 ± 0.69 µg/mL and 47.17 ± 0.58 µg/mL, respectively. All these results indicate that aqueous and ethanol extracts of *S. glauca* leaf display potent anti-inflammatory and antioxidant activities and thus could further be explored for its utility as a therapeutic agent.

Keywords

anti-inflammatory; antioxidant; phytochemical; *Simarouba glauca*

Introduction

Inflammation is an intricate biological process that occurs in response to infection, injury, or other forms of stress, serving as a protective mechanism that aids the body in combating harmful stimuli and facilitating the healing process. It involves the activation of immune cells, which release signaling molecules, such as cytokines, and promote increased blood flow to the affected area. These changes manifest as classic symptoms of inflammation, including swelling, redness, heat, and pain. However, chronic inflammation has been linked to various diseases, including autoimmune disor-

ders, cardiovascular diseases, neurological disorders, cancer, diabetes and rheumatoid arthritis (1).

Oxidative stress is a complex physiological condition that emerges when there is an increase in the production of reactive oxygen species (ROS) coupled with a depletion in the body's antioxidants, leading to a reduced capacity to neutralize these potentially harmful molecules effectively (2). ROS are naturally produced by cellular metabolism, but excessive levels can cause cellular damage, impairing proteins, DNA and other vital components. This damage can contribute to the development and progression of diseases such as cancer and neurological disorders (3). Inflammation and oxidative stress are intricately linked, frequently manifesting concurrently in response to various stimuli or signaling events. Inflammatory processes can induce oxidative stress by enhancing the production of reactive oxygen species (ROS) and compromising the body's antioxidant defense mechanisms (4). Conversely, oxidative stress contributes to inflammation by damaging cells and tissues, which in turn activates the body's inflammatory response. This cycle of damage and inflammation can exacerbate disease progression. Managing oxidative stress is therefore critical in controlling chronic inflammation (5).

In recent scientific advancements in the quest for novel drugs, the medicinal properties of plants have garnered significant attention worldwide, primarily due to their remarkable therapeutic efficacy, the absence of adverse effects and economic viability (6). Plant metabolites, comprising both primary and secondary metabolites, have been extensively explored. Primary metabolites include essential organic compounds such as proteins, glucose, starch, polysaccharides, lipids and nucleic acids. Conversely, secondary metabolites, including terpenoids, alkaloids, flavonoids, saponins, steroids, glycosides, and tannins, exhibit diverse therapeutic effects and have the potential to treat various ailments (7).

Simarouba glauca (S. glauca), belonging to the family Simaroubaceae, is a medicinally significant plant recognized for its bioactive components with therapeutic potential. This versatile evergreen tree, commonly referred to as Shorgum Maram, Tree of Heaven, Dysentery Bark, Paradise Tree, or Lakshmi Taru, is valued for its edible oil and medicinal properties. *S. glauca* is characterized by compound leaves arranged alternately along the stem, with small, fragrant flowers that appear in clusters. It is native to regions including Mexico, South Florida and Central America and is also cultivated in Kenya and various parts of India, particularly in the wastelands of Orissa, Karnataka and Gujarat (8). Indigenous tribes in the areas where *S. glauca* naturally occurs acknowledge the medicinal properties of its leaves. In Panama and Suriname, the leaves have been utilized in traditional medicine to treat respiratory disorders, including coughs and pneumonia. Moreover, they have employed leaf extracts as a febrifuge to treat fever (9, 10). Although the fruits of *S. glauca* are less frequently utilized than other plant parts, they hold significance for indigenous people in countries such as Belize and Costa Rica, who gather the plentiful fruits for consumption. These fruits can be eaten fresh or transformed into jams and preserves, serving as a nutritious resource for local communities (9). The seeds of *S. glauca* are particularly valuable due to their high oil content, which has been historically acknowledged by indigenous tribes in Guatemala and Honduras for its nutritional and therapeutic advantages. The oil extracted from the seeds is rich in essential fatty acids and has been used for food preparation and as a dietary supplement. Additionally, it has been applied directly to promote wound healing and alleviate skin conditions (9, 11, 12).

Pharmacologically, *S. glauca* contains various phytochemical compounds, including alkaloids, terpenoids, quassinoids, saponins, flavonoids, phenolic acids and tannins, which contribute to its efficacy in treating diverse ailments such as dysentery, helminthic infections, malaria, antimicrobial activity, parasitic infections, fever, skin moisturizing, cancer and inflammation (13, 14). However, detailed analysis of phytoconstituents and their biological activity has not been well understood. Thus, our study aims to analyze the phytochemical composition of different extracts obtained from *S. glauca* and to investigate their anti-inflammatory and antioxidant properties.

Materials and Methods

Preparation of crude extracts

The collection of *S. glauca* leaves was made from the gardens of Kuvempu University campus in Jnana Sahyadri, Shimoga, Karnataka, India. After careful washing, the leaves were subjected to a week-long shade-drying process. Once dried, the leaves were crushed and approximately 75 g of the dried leaves were placed carefully into a 500 ml-capacity Soxhlet. Solvents, namely ethanol and ethyl acetate, were used for the extraction process, with a volume of 500 ml for each solvent. The obtained crude extracts were subjected to evaporation using a rotary evaporator. In addition to the solvent extraction, a decoction extraction was carried out using 30 g of *S. glauca* leaves and 300 ml of double distilled water. The samples were evaporated by lyophilization and the obtained residue was carefully stored for further analysis and investigation.

Phytochemical screening of the extracts

The crude aqueous (AQE), ethanol (EE) and ethyl acetate (EAE) of *S. glauca* were obtained and subsequently subjected to phytochemical qualitative tests to analyze the presence of secondary metabolites. The screening involved the assessment of alkaloids, saponins, flavonoids, tannins, terpenoids and carbohydrates using modified procedures based on Harborne's methods (15).

In Vitro anti-inflammatory assays

The anti-inflammatory potential of *S. glauca* leaf extracts was evaluated *in vitro* through various assays. The extracts were evaluated for their potential to prevent the Heat-Induced Hemolysis of healthy human red blood cells, inhibiting albumin denaturation and inhibiting lipoxygenase activity. Dilutions of *S. glauca* leaf extracts from different solvents, ranging from 25 to 600 µg/mL, were prepared using dimethyl sulfoxide (DMSO). We used diclofenac sodi-

um for positive control, which was used at the same concentration as the leaf extracts.

Protection of protein denaturation

A protein denaturation inhibition assay was also used to evaluate the anti-inflammatory properties of *S. glauca* leaf extract. The procedure followed was as described earlier by Sakat *et al*. (16). The crude extracts were dissolved in DMSO in different concentrations from 50 to 600 µg/mL concentrations. A reaction mixture with a final volume of 5 mL was prepared. This mixture was carefully composed by adding 0.2 mL of albumin and 2.8 mL of phosphate-buffered saline with a pH of 6.4. A 2 mL of the respective extract concentration and distilled water of a similar volume were used as control.

The mixture was placed first in an incubator at 37°C for precisely 15 min and then heated at 70°C for only 5 min. As a reference drug, diclofenac sodium was treated similarly. After cooling down, the absorbance of the mixtures was estimated at 660 nm. The following formula was used to determine the percentage of inhibition:

$$\text{Inhibition \%} = \frac{[\text{OD of control} - \text{OD of sample}}{\text{OD of control}]} \times 100$$

Membrane stabilisation method

Preparation of erythrocyte suspension

The suspension of washed human RBC was prepared using the method outlined by previous research (17).. A healthy individual provided whole human blood, which was obtained from the local blood bank. The blood underwent three washes with an isotonic normal saline solution (0.9% NaCl) and was subsequently centrifuged at 3000 rpm for 5 min. Afterwards, the blood that had been washed underwent reconstitution as a 10% (v/v) suspension. This process involved mixing it with an isotonic solution at a pH of 7.4.

Protection of heat-induced hemolysis

This evaluation was carried out by following the procedure outlined by Okoli *et al*. (18). This procedure involved the preparation of a mixture of 0.350 mL of 10% suspension of washed RBC and 0.350 mL of *S. glauca* extract at various concentrations ranging from 50 to 600 µg/mL. Subsequently, the mixture was subjected to heating at 54°C for a duration of 20 min. After incubation, the mixture and control were carefully centrifuged at 2500 rpm for 5 min and the absorbance of supernatant was then detected at 650 nm. The statistical calculation of the percentage of extract inhibition activity was conducted using the following formula.

$$\text{Inhibition \%} = \frac{[\text{OD of control} - \text{OD of sample}}{\text{OD of control}]} \times 100$$

Lipoxygenase inhibitory assay

Lipoxygenase plays a vital role in the metabolic pathway of arachidonic acid, leading to the synthesis of leukotrienes, a mediator contributing to inflammation. Limiting lipoxygenase can reduce leukotriene formation, leading to anti-inflammatory effects (19, 20). To evaluate the LOX inhibition activity of *S. glauca* leaf extracts, in a 96-well plate, 160 µL of 100 mM sodium phosphate buffer (pH 8.0) was mixed with 20 µL of Lipoxygenase solution and10 µL of *S. glauca* leaf extracts and standard (Celecoxib) with different concentrations 6.25-100 µg/mL. The plate was incubated for 10 min. at 25°C, followed by 10 µl of the substrate (Linoleic acid) and measured at 234 nm. The blank was sodium phosphate buffer alone and lipoxygenase with buffer as the control, while the standard control was Celecoxib at different concentrations. The calculation for determining the anti-lipoxygenase activity of the *S. glauca* leaf extracts was performed by using the following formula:

$$\text{Lox inhibition\%} = \frac{[\text{OD of control} - \text{OD of sample}}{\text{OD of control}]} \times 100$$

Determination of antioxidant activity

S. glauca leaf extract (AQE, EE and EAE) was evaluated for its antioxidant capacity. DPPH assay was employed to measure the extract's ability to scavenge free radicals, whereas the ABTS assay focused on assessing its capacity to neutralize ABTS. The extract's potential to reduce ferric ions was determined through the FRAP assay. Additionally, the total antioxidant activity was evaluated using the phosphomolybdate assay, which offered a comprehensive measure of the extract's overall antioxidant potential.

DPPH free radical scavenging activity

In this study, 20 µL of varying concentrations of *S. glauca* leaf extracts and ascorbic acid (standard) from 25 to 200 µg/mL were suspended in 140 µL of the working reagent (6.2 mg of 0.1 mM 1-1-diphenyl-2-picrylhydrazyl in 100 mL ethanol). The mixture's discolouration was detected at 517 nm after 15 min of incubation at 37°C in a dark place (20). The IC_{50} values of the tree leaf extracts and ascorbic acid were established by calculating the percentage at each concentration:

$$\text{\% scavenging} = \frac{[\text{OD of control} - \text{OD of sample}}{\text{OD of control}]} \times 100$$

ABTS free radical scavenging activity

ABTS (2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, as described by Sreeram et al., was used to evaluate the antioxidant properties of *S. glauca* leaf extract. A 7 mM of ABTS was mixed with double distilled water and 2.4 mM potassium persulfate to prepare the ABTS reagent. This mixture was kept in a dark place for 13 to 17 h. The concentrated solution was diluted with methanol and adjusted to 0.7 at 734 nm before analysis (21).

For the analysis, 1 mL of working ABTS reagent was mixed to react with 1 mL of various leaf extracts and ascorbic acid concentrations. The absorbance values were measured using a 96-well microplate reader after 30 min of incubation at room temperature. A control sample with ABTS reagent but without a sample was also used. The IC_{50} values were obtained by plotting the graph of the amount

of ABTS radical inhibition against ascorbic acid. The percentage of the activity of the sample was determined as follows:

$$\% \text{ activity} = \frac{[\text{OD of control} - \text{OD of sample}}{\text{OD of control}]} \times 100$$

Ferric reducing antioxidant power assay

The antioxidant capacity of *S. glauca* leaf extracts was determined using the FRAP method following the procedure outlined by Oyaizu *et al.* (22). *S. glauca* extracts and ascorbic acid at concentrations ranging from 25 µg/mL to 200 µg/mL (1 mL) were mixed with (2.5 mL) phosphate buffer (0.2 mM, pH 7.4) and 2.5 mL potassium ferricyanide (1% W/V). The solution was incubated at 50°C for 20 min. After the incubation, 2.5 mL 10% trichloroacetic acid (TCA) was added and centrifuged at 3000 rpm for 10 min. The clear supernatant (2.5 mL) was gently diluted with the same volume of double distilled water and treated with 0.1% W/V ferrous chloride (0.5 mL). The solution's absorbance was quantified at a wavelength of 700 nm and the antioxidant activity was represented in terms of ascorbic acid equivalent (AAE).

Phosphomolybdate assay

The experimental procedure followed was the established method developed by Prieto *et al*. (23). The phosphomolybdate working reagent was prepared with equal amounts of ammonium molybdate (4 mM), H_2SO_4 (0.6 M) and sodium phosphate (28 mM) were added together. 0.1 ml of various concentrations of *S. glauca* leaf extracts and ascorbic acid was added into one ml of the working reagent. The solution was then kept at a temperature of 95°C for a duration of 90 min. After the incubation period, it was allowed to cool at room temperature, followed by estimating the absorbance of the samples at a wavelength of 695 nm. The total antioxidant capacity of the three extracts has been expressed as an ascorbic acid equivalent (AAE).

Statistical Analysis

The experimental procedures were repeated three times to ensure the reliability of the results and the obtained data were expressed as the mean value accompanied by the standard deviation (M ± SD). Statistical significance was determined using p-values to assess the differences among the groups. Microsoft Office Excel 2021 was employed for data analysis to analyze the statistical significance of the findings.

Results

Phytochemical screening assays

Before conducting bioassays, it is imperative to verify the presence of phytochemicals and secondary metabolites in *S. glauca* leaf extracts. Accordingly, we performed a qualitative analysis to assess the presence of various phytochemicals, as summarized in Table 1. The results indicate the comparative presence of diverse phytoconstituents, including alkaloids, flavonoids, terpenoids, phenols, saponins, tannins, and carbohydrates across three different types of extracts: aqueous extract (AQE), ethanol extract (EE), and ethyl acetate extract (EAE). Ethanol extract (EE) consistently yielded the highest concentrations of flavonoids, terpenoids, and phenols, with moderate to high levels signifying its efficacy in extracting these compounds. This finding aligns with previous research that demonstrated that ethanol is an effective solvent for extracting phenolic compounds from various plant sources, due to its ability to dissolve a wide range of polar and non-polar compounds (24). In contrast, aqueous extracts (AQE) demonstrated elevated levels of saponins and moderate levels of carbohydrates, while ethyl acetate extracts (EAE) revealed a lower presence of saponins and tannins. Furthermore, the findings emphasize the significance of selecting the appropriate solvent for phytochemical extraction, with ethanol proving to be more effective at extracting a broader spectrum of bioactive compounds compared to both aqueous and ethyl acetate solvents.

Table 1. Phytochemical screening tests

Phytoconstituents	AQE	EE	EAE
Alkaloids	+	++	+
Flavonoids	++	+++	+
Terpenoids	++	+++	+
Phenols	++	+++	+
Saponins	+++	-	-
Tannins	++	+	+
Carbohydrates	+	+	+

Phytoconstituent screening test in Aqueous (**AQE**), ethanol (**EE**) and ethyl acetate (**EAE**) extracts. Each phytoconstituent is indicated by "**+**" or "**-**" and its presence by the number of symbols ("**+**" for low, "**++**" for moderate, and "**+++**" for high).

In vitro anti-inflammatory activity

Protection protein denaturation assay

The inhibition of protein denaturation is a method employed to assess anti-inflammatory activity. In this study, we evaluated the ability of *S. glauca* leaf extracts to prevent the denaturation of albumin. The results demonstrate the successful inhibition of albumin denaturation caused by heat. The maximum percentage of inhibition reported was 66.0±1.07%, 65.8±1.27% and 56.5±0.78% for AQE, EE and EAE, respectively. The corresponding IC_{50} values were determined as 328 µg/mL, 342.6 µg/mL and 432.1 µg/mL for AQE, EE and EAE, respectively. Based on the findings,

Table 2. Inhibition of albumin denaturation

Conc. µg/mL	AQE	EE	EAE	Diclof. sodium
50	24.2±0.68^b	17.9±0.24^a	14.0±0.07^a	16.7±0.59
100	29.6±1.10^b	29.5±0.78^b	19.7±0.92	20.5±0.14
200	42.5±0.78	44.4±2.0	30.4±0.86^a	38.7±1.20
400	64.2±1.30^a	61.6±0.70^a	46.4±0.64^b	57.7±1.01
600	66.0±1.07^a	65.8±1.27	56.5±0.78^a	61.6±0.82
IC_{50}	3281 µg/mL	342.61 µg/mL	432.1 µg/mL	369.9 µg/mL

Aqueous (**AQE**), ethanol (**EE**), ethyl acetate (**EAE**), and Diclofenac Sodium results are expressed as mean % ± SD from triplicate measurements, with corresponding IC_{50} values. Significance levels: **a** = p-value < 0.05; **b** = p-value < 0.01; **c** = p-value < 0.0001.

all three extracts of *S. glauca* demonstrated significant potential in preventing albumin denaturation compared to diclofenac sodium, which showed an inhibition of 61.6±0.82% with an IC_{50} value of 369.9 µg/mL (Table 2).

Heat-induced hemolysis

To further elucidate the anti-inflammatory potential of *S. glauca*, we conducted a heat-induced hemolysis assay utilizing the AQE, EE, and EAE leaf extracts. Various concentrations of the extracts, ranging from 50 to 600 µg/mL, were mixed with a suspension of erythrocytes to evaluate their efficacy in preventing heat-induced lysis. AQE, EE and EAE showed 38.95±0.06, 28.6±1.49 and 15.7±0.13% protection with IC_{50} values of 801 µg/mL, 1365 µg/mL and 5047 µg/mL, respectively (Table 3). Under the same concentrations as those of the extracts, the standard diclofenac sodium demonstrated protection of 31.5±0.10% at the maximal dosage and an IC_{50} value of 1308 µg/mL. The data clearly indicates that the *S. glauca* water extract (AQE) has better activity compared to the diclofenac sodium standard as well as other extracts (EE and EAE).

Table 3. Inhibition of heat-inducing hemolysis

Conc. µg/mL	AQE	EE	EAE	Diclof. Sodium
50	18.8 ± 0.06^{b}	10.2 ± 0.20^{c}	7.1 ± 0.18^{b}	14.5 ± 0.58
100	20.3 ± 0.29	12.0 ± 0.17^{c}	11.3 ± 0.18	20.3 ± 0.30
200	27.2 ± 2.37^{a}	15.2 ± 0.29^{c}	11.8 ± 1.16	26.0 ± 0.06
400	34.9 ± 0.09^{c}	20.2 ± 0.69^{b}	14.3 ± 0.13^{c}	29.9 ± 0.35
600	38.95 ± 0.06^{c}	28.6 ± 1.49^{a}	15.7 ± 0.13^{c}	31.5 ± 0.10
IC_{50}	801 µg/mL	1365 µg/mL	5047 µg/mL	1308 µg/mL

Hot-induced hemolysis inhibition was tested with different concentrations of AQE, EE, EAE, and Diclofenac Sodium. Significance levels: **a** = p-value < 0.05; **b** = p-value < 0.01; **c** = p-value < 0.0001.

Lipoxygenase inhibitory assay

The inhibition of lipoxygenase (Lox) activity is another crucial mechanism through which anti-inflammatory agents exert their effects. Our investigation into the lipoxygenase inhibitory activity of various *S. glauca* leaf extracts demonstrated significant dose-dependent inhibition. The maximum inhibitions achieved were 80.3±0.34%, 62.8±0.19% and 67.1±0.09% with AQE, EE and EAE, respectively. Their respective IC_{50} values were measured as 40 µg/mL, 67.1 µg/mL and 64.4 µg/mL. As a standard control, Celecoxib was used, showing a maximum inhibition of 87.5±0.013% and an IC_{50} value of 54.2 µg/mL (Table 4). The data indicate that *S. glauca* AQE exhibits higher inhibitory efficacy compared to both EE and EAE extracts.

Table 4. Inhibition of lipoxygenase

Conc. µg/mL	AQE	EE	EAE	Celecoxib
6.25	13.7±1.0^{a}	7.1±0.29^{a}	6.8±0.09	6.5±0.004
12.5	35.0±0.84^{b}	10.9±0.38^{b}	14.4±0.09	16.0±0.005
25	52.1±1.76^{b}	30.1±0.48^{b}	29.3±1.12	26.7±0.008
50	65.0±0.76^{b}	53.3±0.67^{b}	51.2±0.09^{c}	48.0±0.013
100	80.3±0.34^{b}	62.8±0.19^{c}	67.1±0.09^{c}	87.5±0.013
IC_{50}	40ug/mL	67.1ug/mL	64.4ug/mL	54.2ug/mL

The procedure for inhibiting Lipoxygenase was performed using aqueous (**AQE**), ethanol (**EE**), ethyl acetate (**EAE**) extracts, and celecoxib (standard). Significance levels: **a** = p-value < 0.05; **b** = p-value < 0.01; **c** = p-value < 0.0001.

Antioxidant activity

DPPH free radical scavenging assay

The antioxidant capacity of *S. glauca* extracts was evaluated using the DPPH free radical scavenging assay, a common method for assessing antioxidant potential. Extracts were tested at concentrations ranging from 25 to 200 µg/mL, with ascorbic acid as the standard reference. AQE, EE and EAE showed scavenging rates of 59.3±0.50%, 48.1±1.03% and 12.7±0.01%, respectively. The corresponding IC_{50} values were 180.7 µg/mL for AQE, 209.7 µg/mL for EE, and 678.2 µg/mL for EAE, indicating that EAE had the lowest antioxidant activity. As a comparison, ascorbic acid exhibited a much higher scavenging rate of 80.42±0.85% with an IC_{50} of 103.8 µg/mL (Fig.1). These results suggest that the aqueous and ethanol extracts of *S. glauca* have substantial antioxidant potential, with AQE showing the strongest activity among the extracts tested, though still lower than the ascorbic acid standard.

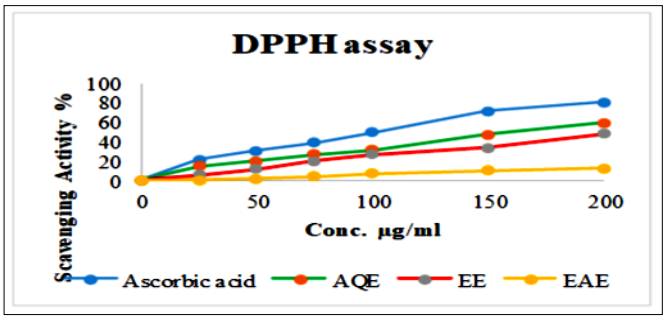

Fig. 1. 2,2-Diphenyl-1-picrylhydrazyl (**DPPH**) radical scavenging activity of the aqueous (**AQE**), ethanol (**EE**), and ethyl acetate (**EAE**) extracts was evaluated in comparison to the control, which utilized ascorbic acid.

ABTS free radical scavenging assay

The ABTS assay provided further insights into the antioxidant capabilities of *S. glauca* extracts. The leaf extracts were tested at various concentrations, along with ascorbic acid (standard) at concentrations of 25, 50, 75, 100, 150, and 200 µg/mL. Among the extracts, the aqueous extract (AQE) displayed the highest scavenging activity, reaching 94.7±1.10%, with an IC_{50} value of 75.8 µg/mL. The ethanolic extract (EE) also showed notable inhibition, with a scavenging activity of 91.0±1.14% and an IC_{50} of 83.9 µg/mL. In comparison, the ethyl acetate extract (EAE) exhibited low-

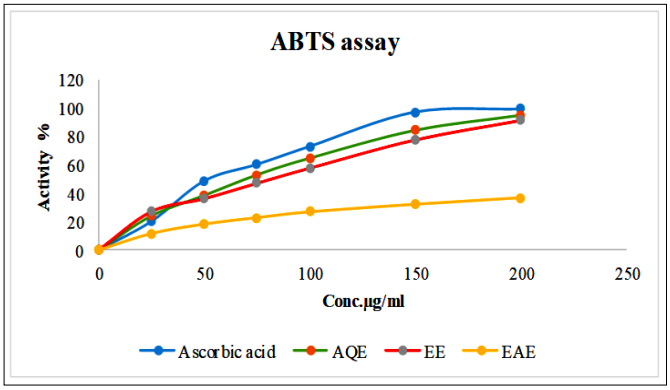

Fig. 2. 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (**ABTS**) antioxidant scavenging activity of aqueous (**AQE**), ethanol (**EE**), and ethyl acetate (**EAE**) extracts, compared to the control of ascorbic acid.

er activity, with 36.7±1.43% scavenging and an IC_{50} value of 225.8 µg/mL. Ascorbic acid, serving as the standard, demonstrated excellent scavenging at the highest concentration, with 99.1±0.79% activity and an IC_{50} of 65.9 µg/mL (Fig. 2). These findings indicate that both the ethanol (EE) and aqueous (AQE) extracts show greater antioxidant potential compared to the ethyl acetate extract (EAE).

Ferric reducing antioxidant power assay

The ferric-reducing power of *S. glauca* extracts was evaluated at concentrations between 25 and 200 µg/mL. Both AQE and EE showed strong reducing capacities, with values of 72.28±0.76 µg/mL and 72.5±0.75 µg/mL, respectively, closely matching the activity of ascorbic acid. In contrast, EAE demonstrated lower activity, measuring 16.8±0.58 µg/mL (Table 5). Notably, all these values were equivalent to the standard ascorbic acid and the outcomes were reported as AAE. These results indicate that AQE and EE possess comparable ferric-reducing abilities to the standard ascorbic acid, while the performance of EAE was considerably lower.

Table 5. Reducing capacity

Conc. µg/ml	AQE	EE	EAE	Ascorbic acid
25	1.37 ± 0.52^{a}	0.2 ± 0.25^{c}	0.03 ± 0.25^{c}	-
50	12.53 ± 0.38^{b}	15.1 ± 0.52	0.4 ± 0.90^{c}	-
75	24.70 ± 0.50^{c}	24.8 ± 0.52^{c}	1.5 ± 0.63^{c}	-
100	33.03 ± 0.52^{b}	34.2 ± 0.25^{a}	3.4 ± 0.38^{a}	-
150	55.53 ± 0.38^{c}	56.6 ± 0.76^{c}	11.6 ± 0.72^{c}	-
200	72.28 ± 0.76^{c}	72.5 ± 0.75^{c}	16.8 ± 0.58^{b}	200 µg/mL

The FRAP analysis for reducing capacity of aqueous (**AQE**), ethanol (**EE**), and ethyl acetate (**EAE**) extracts. The results were expressed as AAE (Ascorbic Acid Equivalence) at a concentration of 200 µg/ml. Significance levels: **a** = p-value < 0.05; **b** = p-value < 0.01; **c** = p-value < 0.0001.

Phosphomolybdate assay

The phosphomolybdate method evaluated the total antioxidant capacity of AQE, EE and EAE extracts from *S. glauca*. At a concentration of 200 µg/mL, EE and AQE exhibited the highest antioxidant potential with values of 80.0 ± 0.87 µg/mL and 81.39 ± 0.69 µg/mL, respectively. Conversely, EAE displayed a lower activity of 47.17 ± 0.58 µg/mL (Table 6). These findings indicate that the crude EE and AQE of *S. glauca* leaf possess significant total antioxidant capacity. The results were represented as AAE. These findings suggest that the crude ethanol (EE) and aqueous (AQE) extracts from *S. glauca* leaves exhibit significant total antioxidant capacity. However, the ethyl acetate extract (EAE) also demonstrated antioxidant capacity, albeit to a lesser extent.

Table 6. Total antioxidant capacity

Conc. µg/mL	AQE	EE	EAE	Ascorbic acid
25	10.67 ± 0.88^{b}	8.61 ± 0.19^{b}	4.06 ± 0.19^{a}	-
50	29.11 ± 0.25^{b}	26.39 ± 0.92^{c}	13.33 ± 0.8^{b}	-
75	37.89 ± 0.42^{a}	35.83 ± 0.51^{b}	16.78 ± 0.97^{b}	-
100	50.22 ± 0.92^{a}	50.28 ± 0.92^{c}	23.22 ± 0.86^{b}	-
150	64.11 ± 0.75^{a}	62.50 ± 0.92^{c}	30.0 ± 0.73^{b}	-
200	80.0 ± 0.87^{a}	81.39 ± 0.69^{c}	47.17 ± 0.58	200 µg/mL

Phosphomolybdate assay for total antioxidant capacity of aqueous (**AQE**), ethanol (**EE**), and ethyl acetate (**EAE**) extracts, and the results were expressed as **AAE** (Ascorbic Acid Equivalence) at a concentration of 200 µg/mL. Significance levels: **a** = p-value < 0.05; **b** = p-value < 0.01; **c** = p-value < 0.0001.

Discussion

The phytochemical screening assays conducted on the leaf extracts of *S. glauca* aqueous (AQE), ethanolic (EE), and ethyl acetate (EAE) yielded significant insights into their secondary metabolite composition. The qualitative analysis revealed the presence of bioactive compounds such as flavonoids, alkaloids, terpenoids, phenols, tannins, and carbohydrates across all three extracts. However, saponins were uniquely identified in the aqueous extract. These findings align with earlier research where similar phytochemicals were reported in *S. glauca* and other medicinal plants, indicating the extract's potential therapeutic applications (24).

For example, flavonoids, widely reported in various studies, exhibit antioxidant, anti-inflammatory, and antimicrobial properties, which have been supported by research on other medicinal plants such as *Moringa oleifera* and *Azadirachta indica* (24). The presence of phenols, which are strong antioxidants, also parallels findings in *Camellia sinensis* extracts, where phenolic compounds contributed significantly to free radical scavenging activities (25).

The identification of saponins exclusively in the aqueous extract is noteworthy, as saponins are recognized for their anti-inflammatory, cholesterol-lowering, and immune-boosting effects. Studies on plants like *Aloe vera* and *Panax ginseng* have similarly identified saponins as key bioactive compounds with broad pharmacological applications. The lack of saponins in ethanolic and ethyl acetate extracts may suggest that these compounds are more water-soluble (26, 27)

In earlier studies, the anti-inflammatory potential of *S. glauca* bark methanolic extract was explored showing notable activity (28). In contrast, our study utilized three solvents with different polarities to obtain ethyl acetate (EAE), ethanol (EE) and aqueous (AQE) extracts. Through various assays, including tests to prevent protein denaturation, heat-induced hemolysis and inhibiting lipoxygenase activity, we analysed the pharmacological potential of these three extracts. In the protein denaturation assay, the extracts exhibited substantial inhibition, with AQE showing the highest inhibition, followed by EE and EAE. Protein denaturation could be used as a marker for various inflammatory and other diseases. Thus, plant extracts such as *S. glauca* have been shown to prevent such protein denaturation and could be a potential candidate for the development of anti-inflammatory drugs. The heat-induced hemolysis assay served as additional validation of the anti -inflammatory capabilities of the extracts. The results indicate that both aqueous (AQE) and ethanol (EE) extracts exhibited significant protective effects against erythrocyte

membrane damage, whereas the ethyl acetate (EAE) extract demonstrated a comparatively weaker efficacy. This finding emphasizes the differential effectiveness of the extracts in mitigating inflammation-induced membrane hemolysis.

Furthermore, this study emphasizes the critical function of protective membrane hemolysis in the modulation of the inflammatory response. By protecting the integrity of cell membranes, this mechanism is instrumental in attenuating inflammation. It accomplishes this by inhibiting the release of lysosomal contents from activated neutrophils, particularly microbial enzymes and proteases (29). These potent mediators, if unleashed, have the potential to exacerbate tissue inflammation and contribute to further cellular damage. Thus, the preservation of membrane integrity plays an essential role in regulating inflammatory processes and has implications in developing therapeutic strategies aimed at combating inflammatory disorders. Lipoxygenase (LOX) plays a crucial role as a rate-limiting enzyme in converting arachidonic acid into leukotrienes (LT), pivotal mediators in the onset of inflammation. Plant extracts have been shown to inhibit LOX, thereby reducing LT levels and exerting an anti-inflammatory effect (30). To further validate the anti-inflammatory potential of *S. glauca*, a lipoxygenase inhibitory assay was conducted. The results demonstrated significant inhibition of LOX activity by all three extracts, AQE, EE and EAE, indicating their capability to attenuate inflammation by suppressing lipoxygenase activity.

Reactive oxygen species (ROS) are well-recognized for their dual role as both destructive agents and essential facilitators of cellular function. These reactive molecules participate in numerous redox-regulating processes that are critical for maintaining cellular homeostasis. However, excessive production of ROS can lead to oxidative stress, a harmful condition characterized by cellular structural damage and the initiation of various pathological conditions. The imbalance between ROS generation and the cellular antioxidant defense system is central to the development of oxidative stress, contributing to the etiology of a wide range of diseases, including neurodegenerative disorders, cardiovascular diseases, and cancer. Thus, while ROS play an indispensable role in normal physiological processes, their dysregulation poses significant threats to cellular integrity and overall health (31). Antioxidants play a crucial role in cellular defense by scavenging free radicals, thereby preventing or mitigating oxidative damage within the body (32). Previous studies have emphasized the antioxidant potential of *Simarouba glauca*, with various plant extracts demonstrating notable efficacy. Specifically, the petroleum ether extract derived from *S. glauca* seeds exhibited significant antioxidant activity, while the methanol extract also showed promising results. Conversely, ethyl acetate extracts demonstrated lower antioxidant activity relative to other extracts, consistent with findings reported in earlier research (28-31).

Our study evaluated the antioxidant potential of *S. glauca* leaf extracts using various assays, including DPPH and ABTS free radical scavenging assays, ferric-reducing antioxidant power assays and phosphomolybdate assays. The results demonstrated that the aqueous and ethanol extracts consistently showcased significant antioxidant capacities compared to the ethyl acetate extract. Specifically, the DPPH assay revealed higher scavenging rates for the aqueous and ethanol extracts, with the ethyl acetate extract displaying the lowest activity. Similarly, in the ABTS assay, the aqueous extract exhibited the most pronounced scavenging activity. These findings are in line with previous research, which highlight the higher antioxidant properties of aqueous and ethanol extracts from various plant sources, attributing this efficacy to the presence of bioactive compounds such as flavonoids and phenolics (33, 34).

Furthermore, the ferric-reducing antioxidant power assay indicated that the aqueous and ethanol extracts had a notable ferric-reducing ability, while the ethyl acetate extract exhibited significantly lower activity. The phosphomolybdate assay corroborated the total antioxidant capacity, revealing that both the aqueous and ethanol extracts possess strong antioxidant potential, whereas the ethyl acetate extract exhibited reduced activity. Polar solvent extracts exhibited significantly improved antioxidant capacities, attributed to their higher yields of phenolic compounds (35).

Conclusion

Several medicinal plants are being widely investigated to combat various acute and chronic inflammatory diseases, as there is an inadequacy of existing therapies to tackle such diseases. The biological activities of plant extracts have been tested by taking various solvents for extraction as well as from different plant parts to identify further potential lead compounds in the process of drug discovery. Here, the primary focus of our study was to investigate the extracts from *Simarouba glauca* in protecting the cell and cellular components by exhibiting antioxidant and anti-inflammatory activities and thus preventing damage by inflammation.

Keeping this in view, we carried out a detailed study of phytochemical evaluation and determined the anti-inflammatory and antioxidant activities of leaf extracts. The preliminary phytochemical analysis of the crude leaf extract has revealed the presence of critical therapeutic principles such as alkaloids, saponins, flavonoids, tannins, etc. Notably, the data clearly indicates the presence of diverse phytochemicals, including saponin, exclusively in AQE, which is known to provide health benefits to individuals in curing various diseases. The AQE and EE demonstrated promising anti-inflammatory and antioxidant properties, making them potential candidates to be used in treatment regimes. Thus, the current findings could be used for further detailed study on these plant extracts to find the exact mechanism of action in possessing various pharmacological properties, especially anti-inflammatory effects. However, further detailed studies are needed in this direction to explore the utility of *Simarouba glauca* total extracts or isolation of its active ingredients to gain

further insight into the mechanism of its action as a natural remedy with therapeutic applications.

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Authors' contributions

WA, RA and NBT did the conceptualization, drafting, writing, supervision and data management. WA and SMA participated in data collection, experiments conducted, data analysis, results and discussion. RA and NBT have done the proofreading, review and corrections.

Compliance with ethical standards

Conflict of interest: The authors have declared that they do not have any conflicts of interest.

Ethical issues: None

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