ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 9, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

COMPARATIVE PHYTOCHEMICAL PROFILE AND ANTIOXIDANT PROPERTY OF BARK, FLOWERS AND LEAVES EXTRACTS OF *SIMAROUBA GLAUCA*

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Received: 20 May 2019, Revised and Accepted: 29 June 2019

ABSTRACT

Objective: The current study was to evaluate and compare the phytochemical constituents and antioxidant activity of bark, flowers and leaves of the tree *Simarouba glauca.*

Methods: The solvent extraction of phytochemicals was carried out using Soxhlet apparatus with ethanol, chloroform, methanol, and water. The antioxidant property was determined by 2,2-Diphenyl-1-picrylhydrazyl, hydrogen peroxide free radical scavenging, reducing power assay, and nitric oxide radical scavenging assay using gallic acid and ascorbic acid as the standards.

Results: The extraction yield was found maximum in the water extract of flower (3.7% w/w). Qualitative and quantitative analysis of phytoconstituents showed that the highest amount of alkaloids and flavonoid content (2.1% w/w) and (3.9% w/w), respectively, was in the chloroform extract of the flower. Phenol and carbohydrate constituent was found to be highest in the methanol extract of leaves 2.5% w/w and 2.2% w/w, respectively. The antioxidant assays showed that the bark possessed maximum antioxidant activity. The water extracts of *S. glauca* bark exhibited scavenging property (90%) with an IC_{50} value 39.63 µg/ml, and the least activity (56%) was observed in the methanol extracts of leaves with an IC_{50} value of 62.96 µg/ml of *S. glauca.*

Conclusion: The study concluded that the water extract of the bark is a potent antioxidant compared to leaves and flowers. Further, *in vivo* studies are essential to enumerate its medicinal use and prove its efficacy in therapeutic applications.

Keywords: *Simarouba glauca*, Solvent extraction, Phytochemical constituents, Antioxidant activity, *In vitro* methods.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i9.34211

INTRODUCTION

Simarouba glauca DC, popularly known as "*Laxmitaru* or Paradise Tree" is an evergreen, edible oil tree incurring great interest as a promising energy crop and for providing therapeutic benefits [1]. This tree is a native of South and Central America, and it is now widely grown in the states of Karnataka, Tamil Nadu, Orissa, Kerala, and Maharashtra [2]. Among several benefits and widespread applications, this tree is becoming a tree of solace for many cancer patients in these states of India [3]. The specific name *Glauca* comes from the Greek word which means blossom intending bluish-green foliage [4]. This multipurpose tree grows to a height of 50 feet with 30 feet spread around, showcased with three-inch-long, shiny, and leathery leaflets which are reddish when young. Once fully grown, it produces a dense crown on top bearing tiny, inconspicuous, yellowish flowers, and tiny clusters of dark purple, one-inch-long, and edible fruits [5]. The leaves and bark extracts have been excessively used as hemostat antimalarial, antidysenteric, anthelmintic, antipyretic and anticancerous [6]. The chief components present in *S. glauca* that is promoting these properties are the quassinoids [7]. The quassinoids present in *S. glauca* are glaucarubin, glaucarubol, glaucarubolone, and the two esters of glaucarubolone, ailanthinone, and glaucarubinone [8]. The quassinoids isolated from the seeds have shown to be effective against *P. falciparum* culture [9,10]. Glaucarubin is found to contain antiamoebic and antiplasmodial activity. Thus, its extracts are commonly used as a medicine to treat gastrointestinal disorders and malaria [9,11]. Glaucarubinone isolated from this tree was found to be responsible for the antileukemic and cytotoxic activities in P388 lymphocytic leukemia model [12,13]. Quassinoids and alkaloids isolated have shown high cytotoxic effect and antimalarial activity. The cytotoxicity is related to the inhibition of protein synthesis [14,15]. In the year 1997, a U.S. patent was filed on *Simarouba* extract of water being able to produce a cosmetic or pharmaceutical product for healthy skin. Today, it is well known that the leaf, seed, bark, and fruit of *S. glauca* have great medicinal properties such as astringent, analgesic, emmenagogue, antiviral, antimicrobial, stomachic, and vermifuge [1,2].

The present study focused on the extraction of phytochemicals using ethanol, methanol, chloroform, and water from bark, flower, and leaves of *S. glauca* and to determine the antioxidant activity of the extracts.

METHODS

Sample collection

Dry leaves, bark, and flower of *S. glauca* were collected separately from the GKVK, University of Agricultural Sciences, Bengaluru. The plant specimen was authenticated from Regional Ayurveda Research Institute for Metabolic disorders, Ref no. RRCBI-mus215. The leaves, flowers and bark were separately washed thoroughly, shade dried at room temperature for 30–40 days. The dried plant samples were pulverized to a powder and stored separately for further analysis.

Preparation of extracts

30 g of shade-dried powder of bark (N1), flowers (N2), and leaves (N3) of *S. glauca* was taken in a Soxhlet apparatus and subjected to the successive solvent extraction process in the order of their polarity. The solvents used were chloroform, ethanol, methanol, and water. The process of extraction using Soxhlet apparatus was carried out in a 250 ml of each solvent separately. The apparatus carrying out the extraction was time framed for complete 48 h after which the solvent mixture was concentrated using a rotary vacuum evaporator [16]. The extracts obtained were

kept in moisture free condition and the samples were used further for phytochemical analysis and to study the antioxidant activity.

Chemicals and reagents

All the organic solvents such as ethanol, chloroform and methanol were obtained from HiMedia, and distilled water was used during the extraction process. Phytochemical screening reagents such as hydrochloric acid (HCl), Mayer's and Wagner's reagent, sulfuric acid (H2SO4), 0.1% ferric chloride (FeCl3), lead acetate (Pb(C2H3O2)2), Fehling's and Benedict's reagent, dil-ammonium hydroxide (NH4OH), and sodium carbonate (Na2CO3), antioxidant assay reagents such as 2,2-diphenyl-1-picryl hydrazyl (DPPH) were all procured from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). 1% hydrogen peroxide (H2O2), 0.1% ethylenediaminetetraacetic acid (EDTA), potassium ferricyanide (K3[Fe(CN)6), 2.5% trichloroacetic acid (CCl3COOH), 0.7% thiobarbituric acid, 0.05 N potassium hydroxide (KOH), 5 mM sodium nitroprusside, 2.5 mM phosphate buffer (pH-7.4), and Griess reagent (1% sulfanilamide, 0.1% N 1-naphthylethylenediamine, and 2% orthophosphoric acid) were procured from Merck, India.

Instrumentation

The absorbance was read using the UV-visible absorption spectroscopy (UV-1800, Shimadzu, Japan). Roteva Equiptronics-Rota evaporator was used to evaporate the solvent under controlled condition.

Phytochemical screening

The phytochemical investigation for individual extracts of *S. glauca* was performed as per the standard protocols [17-19].

Detection of alkaloids

The *S. glauca* sample extracts were dissolved in a test tube containing dilute hydrochloric acid which was later filtered. This filtrate was collected and used to detect the presence of alkaloids through Mayer's test. The obtained yellow precipitate suggested the presence of alkaloids.

Detection of flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colored precipitate indicated the presence of flavonoids.

The sulfuric acid test: Few drops of concentrated H_2SO_4 were added to the plant extracts which formed an orange coloration that evidenced the flavonoid content in them.

Detection of steroids

2 ml of acetic anhydride was taken and to this 5 mg of plant extracts and 2 ml of H_2SO_4 were added. The color changed from violet to bluish green which indicated the presence of the steroid.

Detection of terpenoids

Salkowski's Test: 5 mg of the plant extracts individually were treated with 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was added along the inner surface of a test tube that established a layer. The appearance of reddish-brown color suggested that the terpenoids are present in the extracts.

Detection of anthraquinones

Borntrager's Test: About 5 mg of the plant extracts were treated with 10% HCl and allowed to boil in a water bath for 2–3 min. This was later cooled and filtered. Equal quantity of chloroform was added to the filtrate and a few drops of 10% ammonia were added to the mixture and heated. The pink coloration that developed showed the presence of anthraquinones.

Detection of phenols

Ferric chloride test: 10 mg of extracts were mixed with few drops of $FeCl_3$ solution. Formation of bluish-black liquid showed the phenolic content in the plant extracts.

Lead acetate test: 10 mg extracts were mixed with few drops of lead acetate solution. Formation of yellow-colored precipitate confirmed the phenolic group presence.

Detection of saponins

About 0.5 mg of plant extracts were well stirred after adding 5 ml of distilled water. The froth formation indicated the presence of saponins in the extracts.

Detection of tannins

About 0.5 mg of the extract was diluted using distilled water and incubated in a water bath for a minute. The filtration process was carried out and $FeCl_3$ solution was added to the filtrate. The appearance of a dark green color confirmed the presence of tannins.

Carbohydrate tests

Fehling's Test: Fehling's solution A and B were heated with the 1 ml of the plant extracts, the reducing sugars eventually formed yellowish red colored cuprous oxide precipitate. Hence, the formation of the yellow or brownish-red colored precipitate confirmed the carbohydrates in them.

Benedict's test: Similar to Fehling's test, a free aldehyde group or a keto group present in the reducing sugars reduces the alkaline copper hydroxide to red-colored cuprous oxide. Thus, according to the concentration of sugars, yellowish-red to green color was developed. This confirmed the presence of carbohydrates.

Tests for oil

Grease spot test: About 0.5 mg of the sample extract was put on pieces of paper and a greasy spot penetrating the paper was observed. This happened because lipid does not wet paper, unlike water proving the oil content in extracts.

Quantitative phytochemical analysis

Estimation of alkaloids

1 g of the plant extract was taken in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to it. This was covered and incubated for 4 h at room temperature. Later it was filtered and the plant extract was concentrated up to one-quarter of the initial volume. Ammonium hydroxide was introduced dropwise into the extract until the precipitation was complete. This solution was kept undisturbed and the precipitate was separated, washed using NH_4OH and later filtered again. The residue showed the presence of the alkaloid which was weighed for quantification [18,19].

Estimation of flavonoids

1 g of plant extract was repeatedly extracted using 100 ml of the 80% aqueous methanol. This mixture was filtered using a Whatman filter paper No. 1 and poured into a previously weighed beaker. The filtrate was heated on a water bath and evaporated to dryness, then weighed [20].

Estimation of total phenols

1 g of the plant extracts were made to boil by adding 50 ml of ether for 15 min for the extraction of the phenolic component. 5 ml of the extract was measured and poured into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of NH_4OH solution and 5 ml of pentanol (amyl alcohol) were added into the mixture. The samples were diluted up to 50 ml and kept undisturbed for 30 min for bluish-black color development. The absorbance was read at 505 nm [21].

Estimation of carbohydrates

100 mg of sample extracts were hydrolyzed using hot water in a test tube filled with 5 ml of 2.5 N HCl for a period of 3 h. Later, this was cooled at room temperature and solid Na_2CO_3 was added. The mixture was centrifuged, and the supernatant was separated and made up to 100 ml using distilled water. 1 ml of the diluted solution and 1 ml of phenol were added followed by 5 ml of H_2SO_4. The contents were mixed

well and kept at room temperature for about 20 min. The absorbance of the mixture was measured at 490 nm [22].

Evaluation of antioxidant activity

Antioxidant properties of the plant extracts of *S. glauca* were analyzed by conducting DPPH free radical scavenging assay, hydrogen peroxide assay, reducing power, and nitric oxide (NO) assay as given below [23-29].

DPPH radical scavenging assay

DPPH assay was performed as per Rajakumar *et al.* (1994) method. 1.3 mg/ml DPPH was prepared in HPLC grade methanol of which 75 µl of DPPH solution was utilized, and various concentrations (6.25, 12.5, 50, and 100 µg/ml) of test solutions were prepared, and volume was made up to 3 ml with HPLC grade methanol. All the plant extract samples were compared with the gallic acid which was used as the reference standard. The reaction mixture was well mixed and incubated at room temperature for 15 min, and the absorbance was recorded at 510 nm.

% Scavenged [DPPH] = [(AC−AS)/AC]×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards. Gallic acid was used as standard [23,24].

Hydrogen peroxide scavenging assay

A solution of hydrogen peroxide (4 mM) was prepared in phosphate buffer (pH 7.4). Extracts of concentrations (6.25, 12.5, 50, and 100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml and 4 mM) and final volume was made up to 1.1 ml with distilled water. The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both the extracts and the standard compounds was calculated.

% Scavenged [H_2O_2] = [(AC−AS)/AC]×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards. Gallic acid was used as standard [25].

Estimation of reducing power

The reducing power of the plant extracts was determined by the method stated by Oyaizu *et al.* with slight modifications. The extracts were prepared at varied dilutions such as 6.25, 12.5, 50, and 100 µg/ml and were added to a mixture of 2.5 ml of 20 mM phosphate buffer pH 6.6 and 2.5 ml (1% w/v) potassium ferricyanide and were incubated at 50°C for a period of 30 min. 2.5 ml of (10% w/v) trichloroacetic acid and 0.5 ml of (0.1% w/v) ferric chloride were pipetted into the mixture and incubated again for 10 min at room temperature to obtain a green-colored complex. The absorbance of the color developed was measured at 700 nm using UV-visible spectrophotometer. The stronger absorbance indicated greater reducing power. Gallic acid was used as a positive reference standard [26,27]. The percentage of reducing activity was calculated using the formula.

% Scavenged = [(AC−AS)/AC]×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards.

NO radical scavenging assay

An aqueous solution of sodium nitroprusside at a physiological pH triggers the generation of nitrite oxide instantly which spontaneously binds with an oxygen molecule to produce nitrite ions, which can be detected at 550 nm by spectrophotometer after the addition of Griess reagent. Thus, sodium nitroprusside (5 mM) prepared in standard phosphate buffer saline (0.025 M and pH 7.4) was incubated at 29°C for 3 h in the test tubes after the addition of varied concentration (6.25, 12.5, 50, and 100 µg/ml) of *S. glauca* plant extracts in each of them. For the control, the equivalent volume of buffer was incubated in an identical manner without any extract. 1 ml Griess reagent was added in each of the test samples after incubation and the absorbance of the solutions was measured at 550 nm spectrophotometrically against the blank solution. Ascorbic acid was used as a standard for comparison [28,29]. The free radical scavenging activity was determined by evaluating percentage inhibition as given below.

% Scavenged [NO] = [AC−AS)/AC]×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of extracts or standards.

RESULTS

Extraction yield

The percentage yield obtained after the extraction procedure using Soxhlet apparatus is summarized in Table 1. The N1, N2, and N3 represent to the bark, flowers, and leaves of *S. glauca,* respectively. The solvents used for the extraction process are water, methanol, chloroform, and ethanol which are abbreviated as W, M, C and E respectively. The basic coloration of the extracts was in shades of green to brownish-black. The crude extract yield was found between 0.6% and 3.7% w/w. The percentage yield of chloroform extract of bark and leaves was observed to be 2.4% w/w and 2.7% w/w, respectively, while the overall highest yield was found in the water extract of flower 3.7% w/w. Extraction with the solvents reflected that water has a higher percent of yield followed by chloroform with the three parts of the plant.

Phytochemical profile

Phytochemical screening revealed a wide range of phytochemicals that serves for the possible beneficial traits in the *S. glauca*. So far, many

Table 1: Extraction yield of bark, flowers, and leaves of *Simarouba glauca* with different solvents

Sample	Surface	Color	Extraction yield (% w/w)	Extraction
N1W	Dry	Brown	2.2	Soxhlet
N1M	Oily	Green	0.6	Soxhlet
N1C	Oily	Yellowish-brown	2.4	Soxhlet
N1E	Oily	Yellowish-brown	1.6	Soxhlet
N2W	Dry	Brown	3.7	Soxhlet
N2M	Dry	Grey	1.4	Soxhlet
N2C	Oily	Black	2.1	Soxhlet
N2E	Oily	Yellowish-brown	1.8	Soxhlet
N3W	Dry	Brown	2.2	Soxhlet
N3M	Oily	Brown	1.3	Soxhlet
N3C	Oily	Yellowish-brown	2.7	Soxhlet
N3E	Oily	Yellowish-brown	1.6	Soxhlet

N1W: Water extract of bark, N1M: Methanol extract of bark, N1C: Chloroform extract of bark, N1E: Ethanol extract of bark. N2W: Water extract of flower, N2M: Methanol extract of flower, N2C: Chloroform extract of flower, N2E: Ethanol extract of flower, N3W: Water extract of leaves, N3M: Methanol extract of leaves, N3C: Chloroform extract of leaves and N3E: Ethanol extract of leaves

researchers have reported the presence of alkaloids, tannins, terpenoids, anthraquinones, etc. In the present study, the phytochemicals detected in the samples are represented in Table 2. Water extract of *S. glauca* bark showed the presence of most of the phytochemicals, excluding steroids, terpenoids, and anthraquinones. Methanol extracts of the bark showed the presence of all phytochemicals, excluding flavonoids, steroids, and phenols. The chloroform extract of the bark showed the presence of flavonoids, carbohydrates, and oil. Ethanol extract of bark showed the presence of alkaloid, steroid, saponins, tannins, and oils. Water extract of flower showed alkaloids, phenols, and oil. However, the methanol extract of flowers indicated the absence of alkaloids, flavonoids, and terpenoids. Chloroform flower extracts showed absence of steroids, anthraquinones, and oil. Ethanol extract showed the presence of all the phytochemicals except carbohydrates. Water extract of leaves showed the presence of flavonoids, phenols and tannins. While its methanol extracts showed positive results for all the phytochemicals except flavonoids and terpenoids. The chloroform extract of leaves showed the absence of only steroids. Ethanol extracts of leaves showed the presence of all the phytochemicals analyzed except carbohydrates. Thus, from the phytochemical screening, it was evident that the bark and leaves extracts of *S. glauca* exhibited the maximum number of phytochemicals.

Quantitative analysis of phytochemicals

Alkaloid and flavonoid concentrations in the sample extracts were found to be high compared to phenols and carbohydrates. The chloroform extract of flower sample contained the highest amount of alkaloids (2.1% w/w) and flavonoid content was (3.9% w/w). Phenols and carbohydrates were found to be high in methanolic extracts of leaves sample 2.5% w/w and 2.2% w/w, respectively, as shown in Table 3.

Evaluation of antioxidant activity

DPPH radical scavenging assay

The methanol and water extracts of *S. glauca* bark showed good radical scavenging activity through DPPH assay. Radical scavenger assay depends on the transfer of an electron to the stable free radical DPPH in the presence of the antioxidant. The number of electrons gained is related to a decrease in absorption [30]. The water extract of bark showed maximum scavenging of 80.39% while its methanol extract of showed 78.95% at 100 µg/ml. The IC_{50} values of bark with water and methanol are 38.21 µg/ml and 56.14 µg/ml, respectively. The chloroform extract of flowers showed maximum scavenging property 66.92%, whereas the ethanol extract had 65.12% at 100 µg/ml of the sample extract and the IC_{50} values of are 40.62 µg/ml and 46.07 µg/ml, respectively. In the leaves, maximum activity at 100 µg/ml was 71.14% for the ethanol extract and for methanol extract 63.32% and their IC_{50} values are 39.15 µg/ml and 40.40 µg/ml, respectively. The results are shown in Figs. 1-3 and Table 4.

Hydrogen peroxide scavenging assay

Hydrogen peroxide is a weak oxidizing agent that has permeability to cell membranes and reacts with Fe^{2+} and Cu^{2+} to form hydroxyl radical which leads to cytotoxic effects. Furthermore, hydrogen peroxide possesses the ability to inactivate the thiol (-SH) group in enzymes [31]. The hydrogen peroxide scavenging activity conducted for the sample extracts showed dose-dependent response. Thus, maximum activity was observed at 100 µg/ml (Figs. 4-6). Methanol and water extracts of *S. glauca* bark showed 88.57% and 90.91% scavenging activity, respectively, their corresponding IC_{50} values are 66.46 and 39.63 microgram/ml. Chloroform and ethanol extracts of the flower showed lesser scavenging activity of 69.00% and 66.07%, respectively, at 100 µg/ml, where their IC_{50} values were found to be 47.36 µg/ml for chloroform and for ethanol 54.52 µg/ml. Ethanol and methanol extracts of the leaves showed maximum scavenging property of 75.85% and 63.13%, respectively, at 100 µg/ml, while their corresponding IC_{50} values were observed to be 44.17 µg/ml and 48.29 µg/ml (Table 4).

Reducing power assay

The reducing power is a measure of an antioxidant to donate electrons to the free radicals generated and cause neutralization. The reducing power of depends on the ability of the extracts to reduce ferric cyanide Fe^{3+} to ferrocyanide Fe^{2+} by donating an electron [32]. The results showed that the highest reducing activity is exerted in the bark extracts. The methanol and water extracts of *S. glauca* bark showed 77.72% and 79.25% reducing activity at 100 µg/ml extract concentration, respectively. The maximum reducing capacity of flower extracts of

Table 2: Phytochemical constituents of *Simarouba glauca* extracts

Sample	Alkaloids	Flavonoids	Steroids	Terpenoids	Anthraquinone	Phenols	Saponins	Tannins	Carbo hydrates	Oil
N1W	+	+	−	−	−	+	+	+	+	+
N1M	+	−	−	+	+	−		+	+	+
N1C	−	+		−		−	−		+	+
N1E	+	−	+	−		−	+	+	−	+
N2W	+	−	−	−	−	+	−	−	−	+
N2M	−	−	+	−	+	+		+	+	+
N2C	+	−	−	+	−		+		+	−
N2E	+	−	+	+		+	+	+	−	+
N3W	−	+	−	−	−	+	−	+	−	−
N3M	+	−		−	+	+	+	+	+	+
N3C	+	+	−	+					+	+
N3E	+	+	+	+		+		+	−	+

"+" means positive, "−" means negative and blank indicates slightly positive/negative). N1W: Water extract of bark, N1M: Methanol extract of bark, N1C: Chloroform extract of bark, N1E: Ethanol extract of bark. N2W: Water extract of flower, N2M: Methanol extract of flower, N2C: Chloroform extract of flower and N2E: Ethanol extract of flower. N3W: Water extract of leaves, N3M: Methanol extract of leaves, N3C: Chloroform extract of leaves, N3E: Ethanol extract of leaves

Table 3: Quantitative phytochemicals analysis of sample extracts

Sample	Alkaloids (w/w)	Flavonoids (w/w)	Phenols (w/w)	Carbohydrates (w/w)
N1W	1.1	3.1	0.3	1.9
N1M	1.7	Absent	Absent	1.6
N2C	2.1	3.9	Absent	1.5
N2E	1.4	2.2	2.2	Absent
N3M	1.2	Absent	2.5	2.2
N3E	0.8	1.5	1.8	Absent

N1W: Water extract of bark, N1M: Methanol extract of bark. N2C: Chloroform extract of flower, N2E: Ethanol extract of flower. N3M: Methanol extract of leaves, N3E: Ethanol extract of leaves

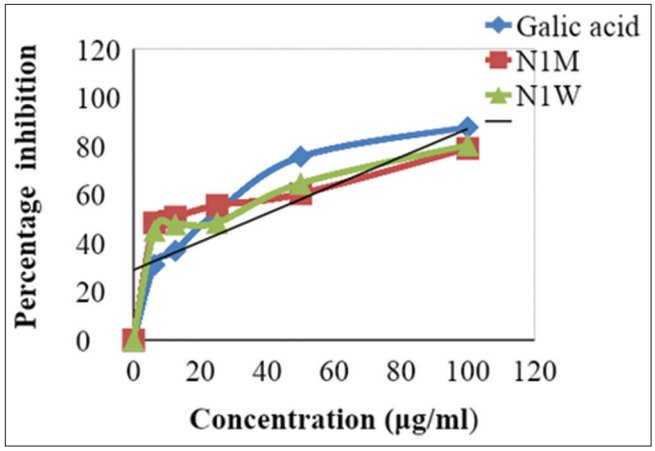

Fig. 1: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of bark extracts of *Simarouba glauca*

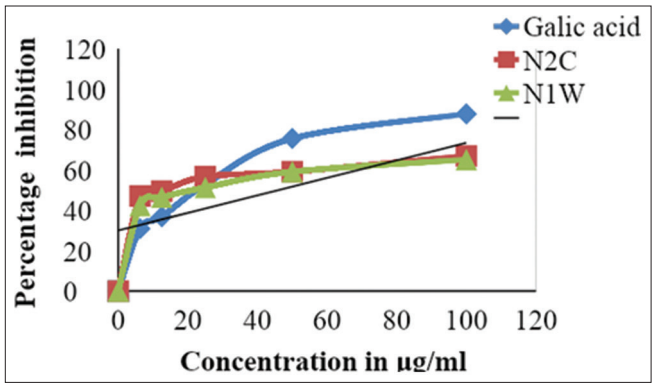

Fig. 2: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of flowers extracts of *Simarouba glauca*

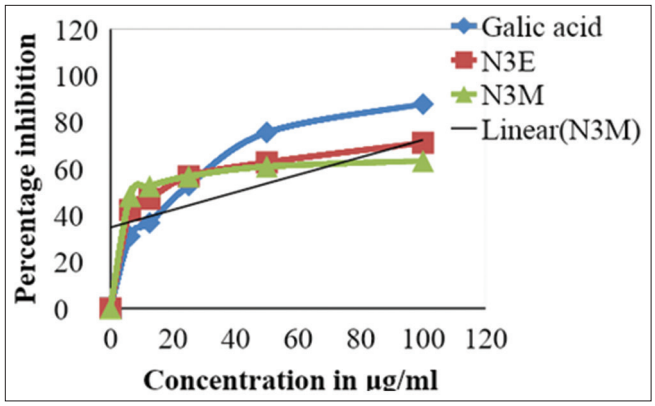

Fig. 3: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of leaves extracts of *Simarouba glauca*

chloroform and ethanol was observed to be 64.98% and 63.07%, respectively, at 100 µg/ml. The ethanol and methanol extracts of leaves showed the activity of 79.44% and 61.16%, respectively, and their corresponding IC_{50} values are 43.50 µg/ml and 46.33 µg/ml (Table 4). Results are represented in Fig. 7-9.

NO radical scavenging assay

NO is a reactive free radical produced by the endothelial cells and phagocytes that yield more reactive species such as peroxynitrite which can be decomposed to form OH radicals. The assay reflected the potential of the plant extracts to suppress the NO released since NO plays a very important role in the pathogenesis of inflammation [33].

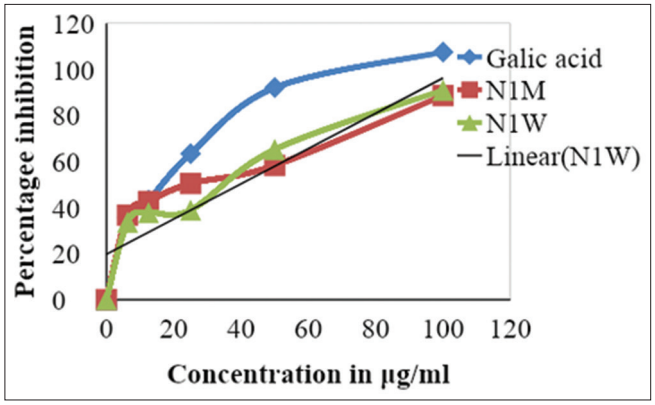

Fig. 4: Hydrogen peroxide radical scavenging activity of bark extract of *Simarouba glauca*

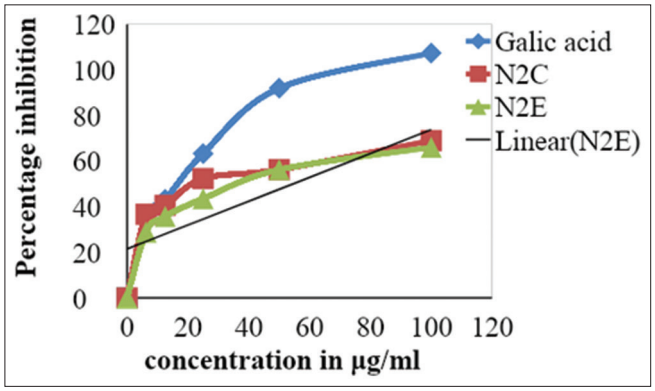

Fig. 5: Hydrogen peroxide radical scavenging activity of flowers extract of *Simarouba glauca*

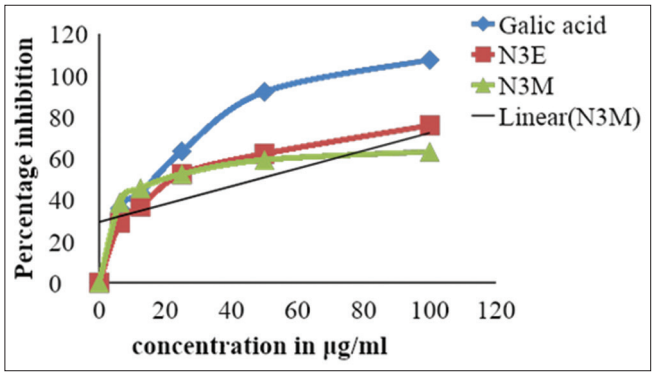

Fig. 6: Hydrogen peroxide radical scavenging activity of leaves extract of *Simarouba glauca*

The assay results of the extracts were compared with the ascorbic acid taken as standard. NO radical scavenging assay showed dose-dependent inhibition; the results are shown in Fig. 10-12 and Table 4. The highest inhibitory activity was observed in the water extract of bark 79.08% at a concentration of 100 µg/ml with 49.12 µg/ml IC_{50} value. Ethanol and chloroform extracts of flowers showed inhibition of nearly 67.0% at concentration 100 µg/ml. The inhibitions exerted by ethanol and methanol extracts of leaves are 77.31% and 56.01%, respectively.

DISCUSSION

Plants are the source of phytochemicals which are found to be bioactive compounds. The plants develop defense mechanism against predators such as insects, herbivores, and microorganisms by producing phytochemicals as secondary metabolites [34]. The

Table 4: Maximum percentage of radical scavenging activity at 100 µg/ml and their respective IC_{50} values of the bark, flowers, and leaves with different solvents

Sample	DPPH		H_2O_2		Reducing power		NO	
	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Gallic acid	87.79	35.30	107.43	24.56	86.64	40.39	-	-
Ascorbic acid	-	-	-	-	-	-	92.02	38.41
N1M	78.95	56.14	88.57	66.46	77.72	62.18	59.15	58.96
N1W	80.39	38.21	90.92	39.63	79.25	39.76	79.08	49.12
N2C	66.92	40.62	69.00	47.36	64.98	45.84	67.01	52.71
N2E	65.12	46.07	66.07	54.52	63.07	51.41	67.02	52.67
N3E	71.14	39.15	75.85	44.17	79.44	43.50	77.31	50.92
N3M	63.32	40.40	63.13	48.29	61.16	46.33	56.01	62.96

N1M: Methanol extract of bark, N1W: Water extract of bark, N2C: Chloroform extract of flower, N2E: Ethanol extract of flower, N3E: Ethanol extract of leaves and N3M: Methanol extract of leaves. DPPH: 2,2-Diphenyl-1-picrylhydrazyl, NO: Nitric oxide

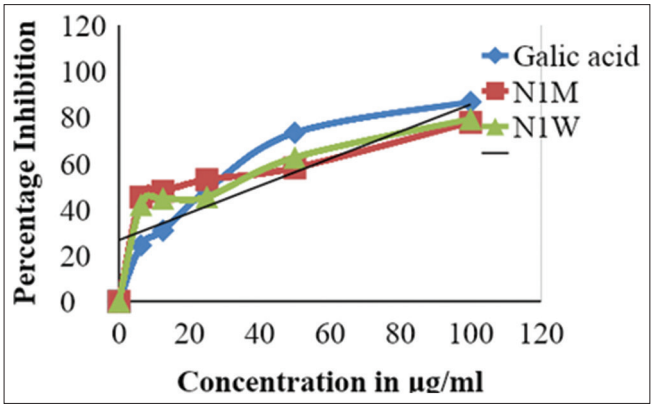

Fig. 7: Reducing the power of bark extracts of *Simarouba glauca*

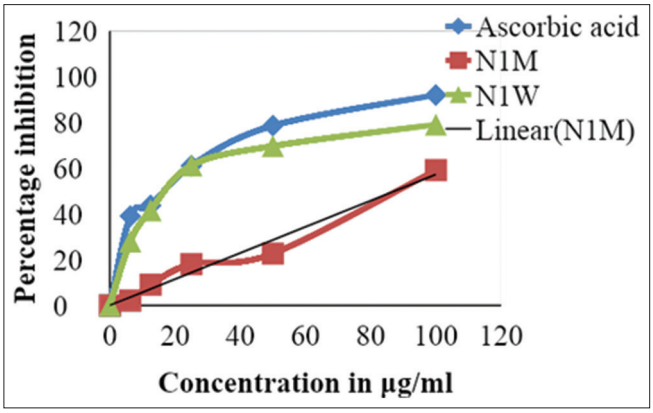

Fig. 10: Nitric oxide scavenging activity of bark extracts of *Simarouba glauca*

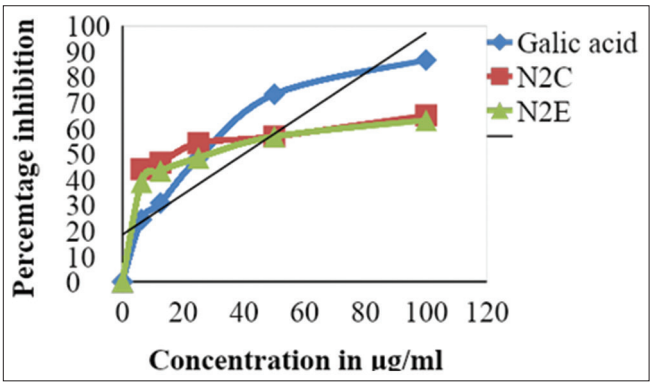

Fig. 8: Reducing the power of flower extracts of *Simarouba glauca*

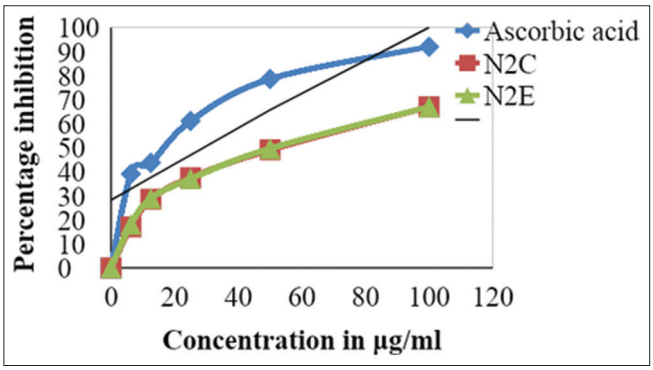

Fig. 11: Nitric oxide scavenging activity of flower extracts of *Simarouba glauca*

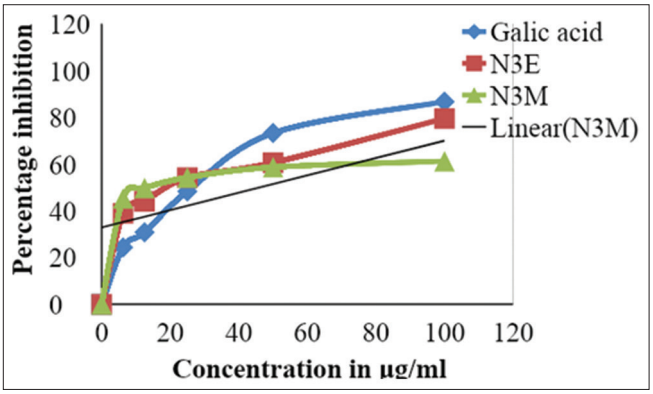

Fig. 9: Reducing the power of leaves extracts of *Simarouba glauca*

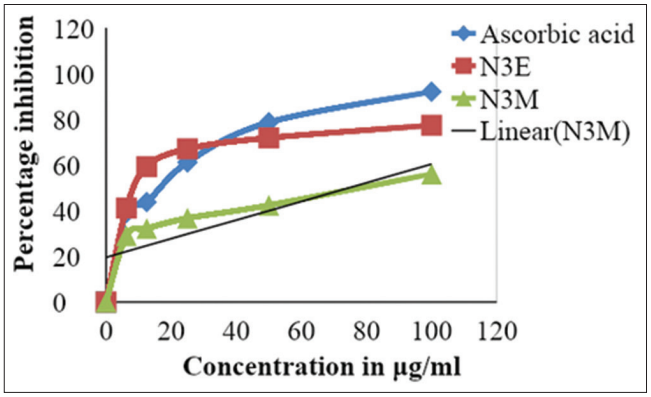

Fig. 12: Nitric oxide scavenging activity of leaves extracts of *Simarouba glauca*

secondary metabolites include the alkaloids, terpenoids, flavonoids, saponins, tannins, and phenols. These compounds are explored for the treatment of many diseases in humans. The quality of phytochemicals is evaluated with its antioxidant capacity [35]. The present research was to investigate and compare the phytochemical constituents and to study the antioxidant activity of bark, flower, and leaves of *S. glauca.* To justify this plant for the medicinal properties it possesses; therefore, it is necessary to evaluate the phytochemical constituents of the plant. Flavonoids and polyphenols are commonly considered as potent antioxidants [36]. The dry powder of bark, flowers, and leaves was subjected to solvent extraction using ethanol, chloroform, methanol, and water. The phytochemical screening showed wide range of the secondary metabolites in these extracts. The results obtained from the phytochemical profiling confirmed the presence of alkaloids, flavonoids, and phenols in the water extract of bark and ethanol extract of flower and leaves. The highest percentage yield was obtained in the water extract of flowers, and the least was found in methanol extract of bark. Quantification of total carbohydrate, phenol, alkaloid, and flavonoid content revealed the presence of high concentration of alkaloid and flavonoid in the chloroform extract of the flower. The effectiveness of phytochemicals in its pure form or a mixture is measured by the antioxidant activity [37]. The antioxidant activity was evaluated by different assays, in methanol and water extract of bark, chloroform and ethanol extract of flowers, and ethanol and methanol extract of leaves. The antioxidant activity of these extracts was measured using the assays: DPPH, hydrogen peroxide, reducing power, and NO. Researchers in the past have explored the phytochemical constituents and antioxidant activity in the leaf extracts of *S. glauca* [38-40]. The *in vitro* studies have further demonstrated the physiological properties of the leaf extract [39]. Anticancer activity of the leaf extract has been shown in the bladder cell lines [40]. A recent study has reported the phytochemical constituents and the antioxidant activity in the root bark [41]. There are no reports on the comparative study of phytochemicals and antioxidant activity of flowers with respect to leaves and bark. The results obtained from the radical scavenging assays revealed that the aqueous extract of bark had maximum scavenging activity irrespective of the solvent used, except for the reducing power. The reducing power of the ethanol extract of leaves was recorded maximum.

CONCLUSION

The results of this study conclude that the bark, flower, and leaves possess the phytoconstituents which is responsible for their active therapeutic properties. Alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, etc., present in any of the bark, flower or leave extracts, suggested a successful qualitative analysis. Antioxidant properties estimated using DPPH, hydrogen peroxide, reducing power, and NO scavenging assays exerted the potent antioxidant property in extracts of bark, flower, and leaves of *S. glauca*. Hence, this plant is a potent source of antioxidant, supporting its medicinal use. The comparative investigation the bark, flowers, and leaves of this plant revealed that the bark has maximum percentage of scavenging activity. Therefore, bark is a better resource antioxidant than flowers and leaves. However, further research through *in vivo* studies on the ability of the bark to act as an antioxidant is essential to comprehend its use in the pharmaceutical industry. The bark can be exploited for the isolation of bioactive compounds having pharmaceutical importance. This can lead to the development of safe drugs against many chronic diseases developed due to oxidative damage.

ACKNOWLEDGMENTS

Authors are grateful to Government Science College (Autonomous), Bengaluru, for the instrumental facility which is procured under DST-FIST (SR-FT/College-272) program.

AUTHORS' CONTRIBUTIONS

Sajeeda and Shivakumar collected the plant sample and performed the phytochemical estimation and antioxidant study. Kolgi and Shivaraj have carried out extraction and phytochemical screening. CS Karigar contributed to the research guidance and coordinated the manuscript writing, editing, and finalization. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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