Research article

A search for anti-inflammatory potential of successive extracts from fruits of Solanum pubescens willd.

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ABSTRACT

Introduction and Aim: Solanum pubescens (S. pubescens) is well known for its therapeutic properties. The current study aimed to conduct phytochemical analyses of various S. pubescens fruit extracts and search for anti-inflammatory and anti-oxidant activities.

Materials and Methods: Qualitative and quantitative phytochemical analyses, in vitro anti-inflammatory and antioxidant activities of Solanum pubescens fruit FHE, FAE1, FAE2, FEE, and FWE extracts at different concentrations (100-500µg/ml) was carried out using standard assays.

Results: Phytochemical studies revealed diverse types of phytoconstituents in different extracts. The FEE extract was observed to be rich in phenolics, whereas the FAE2 and FAE1 extract possessed a significant amount of total flavonoids, while the total tannin was found to be high in FEE. Total alkaloids are found in high amounts in FWE, whereas, FEE extract is rich in saponin and carbohydrates (Table-1). In vitro anti-inflammatory activities at various concentrations of 100-500µg/ml against heat induced hemolysis exhibited 63.82±0.33%, while inhibition of protein denaturation assay revealed 69.63±0.91% activity. Cyclooxygenase and 5-lipoxygenase inhibitory assays showed significant activity of 65.59±1.46% and 74.32±1.68% respectively. Furthermore, in vitro antioxidant activity in DPPH radical scavenging, nitric oxide radicals (NOR), hydroxyl radicals (OH) and superoxide anion (SOR) radical scavenging activities revealed 84.19±0.83%, 60.73±0.79%, 68.6±0.97% and 53.87±1.38% of inhibition respectively.

Conclusion: S. pubescens extracts are found to be rich in bioactive compounds, confirming their potent anti-inflammatory and antioxidant activities, and hence S. pubescens fruit extracts may serve as novel lead for anti-inflammatory compounds.

Keywords: Solanum pubescens; cyclooxygenase; 5-lipoxygenase; anti-inflammation; antioxidant.

INTRODUCTION

Medicinal plants therapeutic value stems mainly on chemical constituents that have a physiological effect on the human body. The most important constituents of medicinal plants are tannins, phenolics, alkaloids, and flavonoids. Recently, most of the research concerned with traditional medicine, looking forward to achieving new principles that lead to the development of improved drugs to treat infectious diseases and other known illnesses (1).

Inflammation is the process that is involved in defending the host against injury and infection. Several diseases, including type 2 diabetes, cancer, neurodegeneration and cardiovascular diseases have recently been identified as having a high inflammatory component (2). The molecular processes that cause inflammation are generally associated with the activities of cells that took part in rearranging and activating tissues. When cells are stimulated by immunological stimuli, pro-inflammatory cells such as monocytes, neutrophils, macrophages, and other host cells become activated and begin to produce molecular mediators that start the inflammatory processes. The most prominent biological markers produced during the inflammatory process are interleukins (IL-1β, IL-6, IL-8), nuclear factors (α and β), tumor necrosis factors (TNF-α), intercellular adhesion molecule-1, induced cyclooxygenase (COX 2), 5-lipoxygenase (5-LOX), prostaglandin E2. The secretion of pro-inflammatory cytokines (TNF, IL-1, and IFN-) and mediators such as histamine, bradykinin, chemokines, are usually the cause of pain in inflammation (2).

Typically, the reactive oxygen species (ROS) causes oxidative stress in cells, which leads to inflammatory and infectious conditions. In phagocytic cells such as mononuclear cells (monocytes, macrophage, lymphocytes) and polymorphonuclear leukocytes (basophils, neutrophils, and eosinophil) that produce a huge amount of different types of ROS such as hydroxyl radical and superoxide anion, that plays a significant role in the host defence mechanism. In addition to their defending effects, excessively
generated ROS disrupt cellular functions, potentially cause cellular and tissue damage via covalent binding and lipid peroxidation, which is followed by tissue injury. This, in turn, increases the inflammatory state. Inflammation is triggered by a variety of stimuli, including physiological and pathological factors, environmental factors, infectious agents, ischemia, antigen-antibody reactions, and free radicals (3, 4).

In the family of Solanaceae, a number of plants exhibited cytotoxic and anti-inflammatory properties. According to recent research, the steroidal alkaloids extracted from Solanum species have substantial cytotoxic and anti-inflammatory characteristics (1). Solanum pubescens is an annual erect wild plant, unarmed shrub reaching 150 cm in height growing as a weed in the forests and hills of South-Eastern Ghats of Andhra Pradesh. It is used in traditional medicines to treat menstrual pain, headache, rheumatoid arthritis, ulcers and tuberculosis, including whooping cough (1).

Furthermore, a wide range of potential therapeutic properties including in the treatment and prevention of hypoglycaemia and treatment of skin infections are attributed to this plant. Anti-inflammatory, anti-depressant, antidiabetic, hepatoprotective, anti-anxiety, myorelaxant, antidiarrheal, and gastroprotective are some of the potential applications of S. pubescens (5). It is imperative to note that all the active principles are usually found in the plant's storage organs like fruits. Taking into account all of the facts, this study is designed to assess the phytochemicals present in various extracts of S. pubescens fruits and to evaluate and confirm the potent anti-inflammatory and antioxidant capacities.

MATERIALS AND METHODS

Medicinal plant identification and collection

The plant material (fruit) was collected from the hill regions of Rayadurg, South-Eastern Ghats, Andhra Pradesh. Referring to the Phytographia (1794) followed by the authentication from Prof. Pullaiah, as a well-known taxonomist, Dept. of Botany, Sri Krishnadevaraya University, Anantapur A.P., India. The fruit was shade dried and a specimen was preserved under herbarium number BTRM012 at Kuvempu University, Dept. of Biotechnology and Bioinformatics, Shankaraghatta, Karnataka-India.

Sequential extraction of plant samples

The unripe fruits were shade dried and then crushed into a fine powder (500 g), using Soxhlet's apparatus they were sequentially extracted in hexane, ethyl acetate, ethanol, and water. To obtain dried extracts, the obtained samples were evaporated at room temperature in a rotary evaporator under low pressure. The dried extracts were designated as follows; fruit hexane extract (FHE), fruit ethyl acetate upper (FEA1), fruit ethyl acetate lower (FEA2), fruit ethanol extract (FEE) and fruit water extract (FWE). The extracts were kept for further studies.

Phytochemical assay of S. pubescens extracts

Phytochemical qualitative analysis

The experimental methods for the various phytochemical screening procedures for secondary metabolites such as flavonoid, tannins, alkaloids, steroids, carbohydrates, proteins, saponins, triterpenoids, coumarins, oils and fats, starch, glycosides were carried out by adopting Harborne's method (6) with some modifications.

Phytochemical quantitative analysis

Total phenol content

The Folin-Ciocalteu method has been used for the determination of the total phenolic content (7). Tests were done in triplicates, and Gallic acid equivalents (GAE) were used to express phenolic compounds.

Flavonoid content

Using the aluminium trichloride method, the flavonoid content was determined and at 435 nm the absorbance was measured. The quercetin equivalent (QE) was utilized to measure the flavonoid content (8).

Tannin content

Sima-Obiang's reference method was used to determine the tannin content (6). Tannic acid was applied as a reference and at 525 nm the absorbance was measured. Tannin content was measured in milligrams of tannic acid equivalent (TAE) per 100 g of extract.

Total alkaloid estimation

The Harborne method was utilized for determining the alkaloid levels (9), 5 g of sample was weighed and 200 ml of 10% acetic acid in C2H5OH in a beaker (250ml) was added and kept for 4 hours. Following filtration, in a water bath, one-quarter of the original volume of the extract was concentrated. Ammonium hydroxide solution was added to the mixture drop by drop until the precipitation was complete. Allowing the entire solution to settle before collecting the precipitates and by using dilute ammonium hydroxide was then washed and filtered and the crude alkaloids obtained were then dried up and weighed.

Saponin content estimation

The Obadoni and Ochuko (6) method was used to determine saponin. In a conical flask 100 ml of 20% aqueous ethanol was added to the sample powder (10 g) and heated at 55°C for 4 hrs in a hot water bath with constant stirring. This was filtered, and the
residues were extracted again with 200 mL of 20% ethanol. The same extract was reduced to 40 ml by keeping it in the water bath at 90°C. For purification, the concentrated extract was shifted to a separating funnel (250ml) while vigorously shaking diethyl ether (20 ml) was added. The purification process has been repeated after recovering the aqueous layer. The extracts combined with n-butanol (60 ml) washed twice using 10 ml of five percent aqueous NaCl, and the collected solvent layer was heated in a water bath and dried, the total content of saponin from fruit extract of S. pubescens was computed as % per gram.

Total carbohydrates
Anthrone method as described by Seifter et al., (6), was used to estimate total carbohydrates. Dextrose was used to prepare standard gradients of 100, 200, 300, 400, and 500µg. 400µl from every extract was taken and concentrated to 1 millilitre with 4 ml of anthrone reagent. For 10 minutes, the reaction mix was kept in a water bath. At 620 nm, the absorbance found red was measured.

Anti-inflammatory assays in vitro
Protein denaturation inhibition
Reaction mixtures of 1 millilitre of S. pubescens extract or standard drug diclofenac sodium in various concentrations ranging from 100-500 µg/ml have been homogenized with 1 millilitre aqueous solution (5%) of bovine serum albumin and left for 15 minutes at 27°C. Distilled water and BSA were utilized to make the control tube. Protein denaturation was achieved by immersing the mix tube for 10 minutes in water bath at 70°C. After cooling to room temperature, the mixture was measured at 660 nm. The test was carried out in triplicate. The percent inhibition was calculated using the formula:

Percentage Inhibition = Absorbance of control − absorbance of sample ÷ absorbance of control ×100 (10).

Membrane stabilization
Suspension of red blood cells (RBCs)
Blood sample has been drawn from a healthy volunteer who had not taken any NSAIDs at least for two weeks before the testing. For 10 minutes, the blood was centrifuged at 3000 rpm. After three washes with normal saline, blood volume was reconstituted as a 10% v/v suspension.

Heat-induced hemolysis
The reaction mixture contained 1 ml of 10% RBC suspension and test sample 1 ml at various concentrations ranging from 100 to 500µg/ml, with saline serving as a control instead of the test sample. Diclofenac sodium was utilized as a standard medication. The tubes were kept for incubation in a water bath at 56 °C for 30 minutes. The reaction mixture was allowed to cool and then centrifuged at 2500 rpm for 5 minutes and the supernatants were measured at 560 nm absorbance. This test was triplicated. The following formula was applied to calculate the % haemolysis inhibition:

\[ \text{Inhibition} \% = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}} \] (11).

Inhibition of cyclooxygenase and 5-lipoxygenase assay
Preparation of lymphocyte cultures: Human peripheral blood lymphocytes were cultured using RPMI 1640 medium (HIMEDIA) with inactivated fetal calf serum 20%, streptomycin, and penicillin. Phyto-hemagglutinin (HIMEDIA) was used to stimulate cell proliferation. Adding plasma (1 × 10^6 cells/ml) and the incubation for 72 hours after filtration with sartorius filter (0.2-µm pore cellulose acetate filter). The culture was activated by adding 1µl of lipopolysaccharide and incubating it for 24 hours, to this diclofenac sodium and S. pubescens fruit extracts were added at different concentrations (100-500µg/ml). After 24 hrs of incubation the culture were centrifuged for 10 min at 6000 rpm. Supernatant was removed and 50µl of cell lysis buffer was added to the cell residue and re-centrifuged for 10 minutes at 6000 rpm. Viji and Helen (12) described method was used to test the anti-inflammation.

Cyclooxygenase assay
The assay was carried out using haemoglobin, tris HCl buffer, glutathione mixture and enzyme. The mixture was incubated for 20 minutes at 37°C after adding the arachidonic acid and TCA (10% in 1N HCl, 0.2 ml). 0.2 ml of TBA was added into the mixture, and then heated for 20 minutes in boiling water followed by centrifugation for 3 minutes at 1000 rpm in order to measure the activity of COX at 632 nm using the supernatants.

Assay of 5-lipoxygenase
Linoleic acid (70mg) was pipetted into 4 ml of non-oxygenated water with 70mg of interpolation, then (0.5 N) sodium hydroxide and 25 mL of distilled water were added. The finished mixture was divided into 0.5 mL tubes, nitrogen washed, and frozen. The reaction was carried out in a 25°C quartz cuvette with a 1cm optical path. Absorbance was evaluated at 234 nm of reaction mixture containing 2.75 ml of tris buffer (pH 7.4), 50 ml enzyme and 0.2 ml sodium linoleate (12). To calculate % inhibition, the following formula was used:

\[ \text{Inhibition} \% = \frac{\{\text{abs control} - \text{abs sample}\} \times 100}{\text{abs control}} \]
In vitro antioxidant activity

DPPH free radical scavenging

To determine the DPPH free radical scavenging activity of hexane, ethyl acetate, ethanol and water extracts of S. pubescens with different concentrations (100µg, 200µg, 300µg, 400µg and 500µg/ml) Brand-Williams et al. (1995) method was followed with minor changes (13, 14). The absorbance of stable DPPH was evaluated at 517 nm and as a standard, BHT (20µg, 40µg, 60µg, 80µg and 100µg) was used as a standard. The following formula: scavenging activity (%) = Absorbance of test/Absorbance of blank × 100 was used to calculate % inhibition. IC$_{50}$ was evaluated by the equation: IC$_{50}$ = $\frac{x_50 - x}{\Sigma}$, where the sum of extracts concentrations is $\Sigma C$, and at different concentrations $\Sigma$ is the aggregate of level inhibition.

Hydroxyl radical scavenging

The scavenging activity of hydroxyl radicals was determined by the modified protocol (13). 200 µl of 10 mM F$_2$SO$_4$, 7H$_2$O, 200 µl of 10 mM EDTA, and 200 µl of 10 mM 2-deoxyribose were used in the Fenton reaction mixture and mixed with 0.1 M phosphate cradle (1.2 ml, pH 7.4) containing 500 µl of extracts at various concentrations of (100µg, 200µg, 300µg, 400µg, and 500µg/ml). Prior to incubation for 4 h at 37°C, 200 µl of 10 mM H$_2$O$_2$ was added to the mixture. Later, 1000 µl of 2.8% TCA and 1000 µl of 1% TBA were added and the mixture was kept for 10 minutes in a water bath for boiling. After cooling the tubes at room temperature, they were placed for centrifugation at 2000 rpm for 5 minutes. The absorbance was measured at 532 nm. BHT (20µg, 40µg, 60µg, 80µg and 100µg) was used as standard and the above equations were used to compare the hydroxyl radical scavenging activity level and IC$_{50}$ of the extracts and the standard.

Superoxide anion scavenging activity

The Nishimiki (15) method was used for the determination of superoxide anion scavenging efficacy. In water, 1 ml of NBT (pH 7.4), 1 ml NADH (pH 7.4) and 100 µl ethanol extract were mixed with water to begin the reaction, PMS solution 100 µl (60 M PMS in 100 mM phosphate buffer pH maintained at 7.4) was added. After 5 minutes of incubation at 25°C the absorbance at 560 nm was measured and compared with a blank. BHT (20µg, 40µg, 60µg, 80µg and 100µg) was used as the standard. Increased superoxide anion scavenging activity was indicated by lower absorbance in the reaction mixture.

Nitric oxide radical scavenging

In this assay a 3 ml reaction mixture containing 2 ml of 10 mM sodium nitroprusside, phosphate buffer saline 500 µl (pH 7.4, 0.01 M), and 500µl of plant extract was incubated for 150 minutes at 25°C. Reaction mixture of (500 µl) when combined with 1000µl of C$_2$H$_5$H$_2$NO$_2$S reagent (to 20% glacial acetic acid, sulfanilic acid, 0.33 percent) and kept for 5 minutes to allow the diazotization reaction to complete. To this mixture 1 ml of naphthyl ethylene diamine dihydrochloride (0.1 percent) was added and allowed to stand in diffused light for 30 minutes before being read at 540 nm against a blank. The extract's scavenging capacity was compared to that of standard BHT (20µg, 40µg, 60µg, 80µg and 100µg) (13). The level of nitric oxide radical scavenging activity was calculated by following the above equations.

RESULTS

Phytochemical screening

Phytochemical analysis revealed that S. pubescens fruit extracts are high in secondary metabolite compounds (flavonoids, tannins, saponins, steroids, alkaloids, carbohydrates, fats and oils etc.), while terpenoids were found in the ethyl acetate extract only (Table 1; Fig.1).

To identify major chemical groups, extracts were quantitatively screened. Table-2 shows the total phenolics, flavonoids, carbohydrates, tannins and alkaloid contents. The phenolic content of fruit extracts was calculated, where ethanol extract observed to be the richest in phenolics, followed by ethyl acetate extract lower fraction (FAE2), water extract, ethyl acetate extracts upper fraction (FAE1) and hexane extract, amounting to 192.39 ± 1.25, 146.56 ± 1.2, 111.85 ± 0.74, 69.84 ± 0.65 and 91.56 ± 0.86 mg/g, respectively.

The results of the total flavonoids showed a significant amount in ethyl acetate (FAE2 101.82±1.08 mg/g and FAE1 81.12±1.14 mg/g), followed by ethanol extract (83.18±1.42 mg/g), water extract (85.55±0.85 mg/g) and n-hexane extract (75.15±0.57mg/g). The amount of tannin was the highest in the ethanol extract with 188.26±0.38 mg/100g of extract.

Further, total alkaloids and saponins were quantified using a direct method. The water extract (75.9±0.67 mg/g) is found to be high in alkaloids followed by ethyl acetate extracts (FAE1 64.85±0.65 mg/g and FAE2 51.05±0.47 mg/g), and ethanol extract 46.73±0.68 mg/g respectively. Interestingly, among the fruit extracts only ethyl acetate and ethanol were high in saponins where the ethanol extract showed high amounts (Table 1).

The carbohydrate quantification revealed that ethanol extract contains approximately 720.2±1.19 mg/g of carbohydrates, ethyl acetate extracts with FEA1 620.41±0.86 mg/g, and FEA2 291.42±0.64 mg/g.
water and hexane extracts containing, 543.13±0.61, 237.37±1.29 mg/g respectively (Table 1).

**Table 1: Quantitative evaluation of the S. pubescence extracts**

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>FHE</th>
<th>FAE1</th>
<th>FAE2</th>
<th>FEE</th>
<th>FWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>75.15±0.57</td>
<td>81.12±1.14</td>
<td>101.82±1.08</td>
<td>83.18±1.42</td>
<td>85.55±0.85</td>
</tr>
<tr>
<td>Phenolics</td>
<td>91.56±0.86</td>
<td>96.84±0.65</td>
<td>146.56±1.2</td>
<td>192.39±1.25</td>
<td>111.85±0.74</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>237.37±1.29</td>
<td>620.41±0.86</td>
<td>291.42±0.64</td>
<td>720.2±1.19</td>
<td>543.13±0.61</td>
</tr>
<tr>
<td>Tannin</td>
<td>Nil</td>
<td>53.39±1.29</td>
<td>171±0.96</td>
<td>188.2±0.38</td>
<td>175.86±0.64</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Nil</td>
<td>59.84±0.43</td>
<td>88.62±0.5</td>
<td>Nil</td>
<td>74.6±0.45</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Nil</td>
<td>64.85±0.65</td>
<td>51.05±0.47</td>
<td>46.73±0.68</td>
<td>75.9±0.67</td>
</tr>
<tr>
<td>Saponins</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>53.38±0.74</td>
</tr>
</tbody>
</table>


**Heat induced haemolysis**

All the extracts of *S. pubescens* effectively inhibited heat-induced haemolysis. Plant extracts illustrated that at various concentrations of 100, 200, 300, 400 and 500µg/ml protect the erythrocyte membrane significantly from heat-induced lysis, whereas FAE1 showed protection of 63.82±0.33% followed by FWE 62.35±0.86%, FAE2 58.72±0.22% and FEE 58.2±0.29% (Fig. 2). Diclofenac sodium 100µg/ml used as standard significantly reduced the damaging effects of heat against haemolysis.

**Inhibition of protein denaturation**

The *S. pubescens* extracts effectively inhibit heat-induced protein denaturation (albumin). The significant inhibition of ethyl acetate upper extract (FAE1) showed 69.63±0.91% and ethyl acetate lower (FAE2) 64.4±1.39% activity followed by water extract (FWE) with 55.5±0.91%, ethanol extract (FEE) with 36.13±1.39%. Hexane extract (FHE) showed minimal inhibition of 27.23±0.91% at the concentration of 500µg/ml, whereas Diclofenac sodium produced 71.73±1.39 % inhibition (Fig. 3).
Cyclooxygenase inhibitory assay

The impact of S. pubescens extracts over prostaglandin production was determined using cyclooxygenase activity estimation at different concentrations between 100-500 μg/ml. The results showed that ethyl acetate (FAE1 and FAE2) with highest inhibition of 68.99±1.05%, 65.59±1.46%, ethanol (FEE) and water (FWE) extracts with inhibition rates of 61.34±1.07% and 60.73±1.42% respectively. n-hexane (FHE) inhibition was found to be low at the rate of 36.84±1.26% significantly inhibiting the activity of cyclooxygenase as compared to diclofenac sodium which is represented in Fig. 4.

![Cyclooxygenase inhibition assay](image)

**Fig. 4:** Inhibition of cyclooxygenase (%) by solvent extracts.

Lipoxygenase inhibitory assay

The effect of various S. pubescens extracts on leukotriene production was studied using 5-lipoxygenase activity (Fig. 5).

![5-lipoxygenase inhibition assay](image)

**Fig. 5:** Inhibition of 5-lipoxygenase (%) by solvent extracts.

The results of S. pubescens extracts, showed high inhibition activity among ethyl acetate (FAE1; 67.57±1.00 and FAE2; 74.32±1.68) followed by ethanol (FEE; 64.86±1.35) water (FWE; 63.9±1.17) and n-hexane (FHE; 43.05±0.84) extracts. Diclofenac sodium was used as a standard (Fig. 5).

**In vitro antioxidant activity**

The results of various S. pubescens fruit extracts on DPPH inhibition are summarized in Table 2. Ethyl acetate extracts (FAE1 and FAE2) were the most effective DPPH radical scavengers (IC₅₀ value of 43.44±1.12 and 37.81±0.23 μg/ml) with the inhibition of 84.19±0.83% and 78.08±0.96% followed by ethanol extracts (FEE) where it showed good inhibition at 76.06±0.11% (IC₅₀ value of 42.54±1.73). The water extract (FWE) was also found to be a good radical scavenger with the inhibition of 66.28±1.75% (IC₅₀ value of 43.44±1.12). The reference standard diclofenac exhibited stronger activity with an IC₅₀ value of 81.79±67μg/ml). Similarly, the ethyl acetate extracts showed significant nitric oxide radical, hydroxyl radical, and superoxide radical scavenging activities (Table 3, 4 and 5). Water extract at various concentrations is observed to demonstrate 64.59±1.14%, 61.34±1.07% and 51.85±0.6% activity respectively in the said assays (Table 3, 4 and 5). Similarly, ethanol extract showed significant activity of 61.8±0.81%, 65.61±0.49% and 50±0.77%, while n-hexane was found to be very weak in the activity.

**DISCUSSION**

In the current study, phytochemical screening and *in vitro* anti-inflammatory activity of S. pubescens fruit extracts were investigated. It is well established that phytochemical constituents are primarily responsible for the pharmacological effects of individual plants. Plants that contain various types of phytochemicals have a long history of serving people all over the world against various elements (16). In this study, plant fruit samples were subjected for extraction with various organic solvents such as, n-hexane, ethyl acetate, ethanol, and water. Phytochemical screening of S. pubescens was carried out in order to discover beneficial phytochemical constituents with potential activities. This investigation suggests the presence of phytochemical metabolites, such as alkaloids, flavonoids, terpenoids, saponins and tannins in the various extracts isolated from S. pubescens (Fig. 1). The quantitative phytochemical screening revealed significant amounts of phytochemicals as shown in table-1. To determine the relationship between the phytochemical constituents and pharmacological efficacy found in the plant’s extracts, experiments were conducted to assess the free radical scavenging capacity and anti-inflammatory activity.

Antioxidants can prevent the oxidation of other molecules and thus exert beneficial effects in the prevention of degenerative illness. Free radicals, in particular, are thought to act as key agents in a variety of long-term pathologies, including inflammation and cancer, and are also linked to the ageing process (17). In this study, DPPH inhibition, nitric oxide radical, hydroxyl (OH) radical, and superoxide radical scavenging methods were used. The findings showed that the S. pubescens extracts provided significant antioxidant protection against the damaging effects of free radicals. The effect exhibited by the extracts is very similar to that of the standard drug used. The ability of S. pubescens fruit extracts to scavenge free radicals was confirmed.
radicals by exhibiting antioxidant activity may be attributed to the presence of high phenolic content in the extracts (Table 1; 18-20). The established free radical scavenging efficacy is certainly playing an important role in the anti-inflammatory process.

Table 2: The impact of S. pubescens fruit extracts on DPPH inhibition

<table>
<thead>
<tr>
<th>Dose(μg/ml)</th>
<th>FHE</th>
<th>FAE1</th>
<th>FAE2</th>
<th>FEE</th>
<th>FWE</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>11.32±0.2</td>
<td>35.30±1.03</td>
<td>39.30±0.20</td>
<td>23.87±0.92</td>
<td>34.82±1.21</td>
<td>49.76±1.32</td>
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<tr>
<td>200</td>
<td>19.44±0.59</td>
<td>38.00±0.39</td>
<td>64.02±1.34</td>
<td>53.12±0.89</td>
<td>49.37±1.37</td>
<td>53.39±0.78</td>
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<tr>
<td>300</td>
<td>27.11±0.39</td>
<td>49.81±1.74</td>
<td>70.78±0.96</td>
<td>62.15±1.05</td>
<td>58.06±0.85</td>
<td>55.63±0.92</td>
</tr>
<tr>
<td>400</td>
<td>30.02±0.83</td>
<td>65.25±1.17</td>
<td>75.15±0.40</td>
<td>74.22±0.41</td>
<td>61.11±0.15</td>
<td>59.35±1.06</td>
</tr>
<tr>
<td>500</td>
<td>40.19±0.52</td>
<td>84.19±0.83</td>
<td>78.08±0.39</td>
<td>76.06±0.11</td>
<td>66.28±1.75</td>
<td>63.07±1.04</td>
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</table>

Table 3: The impact of S. pubescens fruit extracts on nitric oxide radical (NO•) scavenging activity

<table>
<thead>
<tr>
<th>Dose(μg/ml)</th>
<th>FHE</th>
<th>FAE1</th>
<th>FAE2</th>
<th>FEE</th>
<th>FWE</th>
<th>STANDARD</th>
</tr>
</thead>
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<tr>
<td>100</td>
<td>30.14±0.79</td>
<td>39.27±1.15</td>
<td>40.03±0.66</td>
<td>43.23±1.10</td>
<td>47.49±1.05</td>
<td>36.82±1.03</td>
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<tr>
<td>200</td>
<td>35.16±0.70</td>
<td>43.07±1.07</td>
<td>43.53±0.81</td>
<td>46.27±0.40</td>
<td>56.16±1.21</td>
<td>41.93±0.87</td>
</tr>
<tr>
<td>300</td>
<td>45.21±1.21</td>
<td>47.64±1.07</td>
<td>47.64±0.66</td>
<td>51.6±0.70</td>
<td>59.21±0.40</td>
<td>46.73±1.06</td>
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<tr>
<td>400</td>
<td>63.77±0.66</td>
<td>55.86±1.07</td>
<td>58.3±1.00</td>
<td>61.04±0.55</td>
<td>60.27±0.53</td>
<td>49.36±0.88</td>
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<tr>
<td>500</td>
<td>59.97±1.10</td>
<td>60.73±0.79</td>
<td>60.88±0.93</td>
<td>61.8±0.81</td>
<td>61.34±1.07</td>
<td>52.05±0.53</td>
</tr>
</tbody>
</table>

Table 4: The impact of S. pubescens fruit extracts on hydroxyl (OH) radical

<table>
<thead>
<tr>
<th>Dose(μg/ml)</th>
<th>FHE</th>
<th>FAE1</th>
<th>FAE2</th>
<th>FEE</th>
<th>FWE</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.93±0.49</td>
<td>46.54±1.14</td>
<td>45.42±1.14</td>
<td>42.24±0.65</td>
<td>44.11±0.81</td>
<td>48.53±0.75</td>
</tr>
<tr>
<td>200</td>
<td>6.54±1.16</td>
<td>54.39±0.81</td>
<td>48.97±0.65</td>
<td>48.22±1.04</td>
<td>47.85±0.86</td>
<td>55.23±1.06</td>
</tr>
<tr>
<td>300</td>
<td>12.34±1.18</td>
<td>60.37±0.89</td>
<td>57.38±1.48</td>
<td>49.91±0.81</td>
<td>52.34±1.17</td>
<td>59.96±0.85</td>
</tr>
<tr>
<td>400</td>
<td>18.32±0.99</td>
<td>66.36±1.17</td>
<td>63.36±1.31</td>
<td>59.63±1.17</td>
<td>59.07±0.97</td>
<td>60.46±0.95</td>
</tr>
<tr>
<td>500</td>
<td>22.80±0.99</td>
<td>68.62±0.97</td>
<td>67.29±0.99</td>
<td>65.61±0.49</td>
<td>64.49±1.14</td>
<td>65.85±1.04</td>
</tr>
</tbody>
</table>

Table 5: The impact of S. pubescens fruit extracts on superoxide radical

<table>
<thead>
<tr>
<th>Dose (μg/ml)</th>
<th>FHE</th>
<th>FAE1</th>
<th>FAE2</th>
<th>FEE</th>
<th>FWE</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.37±0.45</td>
<td>33.67±0.61</td>
<td>32.32±0.87</td>
<td>33.67±1.02</td>
<td>30.98±1.31</td>
<td>21.06±0.69</td>
</tr>
<tr>
<td>200</td>
<td>6.57±0.51</td>
<td>35.35±0.19</td>
<td>33.84±0.58</td>
<td>36.36±0.87</td>
<td>32.83±0.77</td>
<td>23.94±1.05</td>
</tr>
<tr>
<td>300</td>
<td>9.6±0.87</td>
<td>37.88±1.34</td>
<td>39.39±1.05</td>
<td>42.09±1.10</td>
<td>36.87±1.05</td>
<td>26.53±0.87</td>
</tr>
<tr>
<td>400</td>
<td>15.32±1.18</td>
<td>47.64±0.61</td>
<td>46.8±1.18</td>
<td>45.62±1.21</td>
<td>46.8±0.61</td>
<td>29.32±1.04</td>
</tr>
<tr>
<td>500</td>
<td>16.67±1.05</td>
<td>53.87±1.38</td>
<td>50.51±0.77</td>
<td>50±0.77</td>
<td>51.85±0.61</td>
<td>38.99±1.50</td>
</tr>
</tbody>
</table>

In the current study ethyl acetate, ethanol, water, and n-hexane extracts showed good anti-inflammatory activities (Table 2). These findings are in agreement with the efficacies exhibited by the related species of genus solanum which can be mainly attributed to the presence of phenolic compounds and flavonoids such as quercetin which is reported to have anti-inflammatory properties (21, 22). Furthermore, the in vitro anti-inflammatory assays have demonstrated potent abilities of plant extracts. It is well demonstrated that when red blood cells (RBCs) are exposed to harmful substances such as heat, the membranes lyse, resulting in haemolysis and oxidation of haemoglobin. The denaturation of proteins is a well-known cause of inflammation (23, 24). It is presumed that heat-induced lysis of the human erythrocyte membrane was prevented by S. pubescens extract at different concentrations ranging between 100-500 μg/ml (Fig. 2) simultaneously reducing protein denaturation (Fig. 3). The stabilization of RBC membranes suggests that S. pubescens fruit extracts might have been involved in this process and in turn prevented protein denaturation and thus exhibiting anti-inflammatory properties.

Further, S. pubescens extract against COX-2 and LOX showed potential anti-inflammatory activity (Fig.4, 5) exhibiting significant reduction in prostaglandin production, which confers excellent anti-inflammatory properties of the extracts. Substances that can inhibit both COX and LOX, resulting in a significant lessening of the prostaglandin production, produce a wide range of anti-inflammatory activities and are thought to have outstanding pharmacological safety profile in clinical practice (10).

CONCLUSION

Thus, it is evident from these findings that S. pubescens exhibit very strong antioxidant and anti-inflammatory properties. These findings have established the fact that S. pubescens which is effectively being used in the traditional medicine for...
the treatment of chronic inflammatory diseases do possess the antioxidant and anti-inflammatory properties. Hence, this plant may serve as a potential source to isolate new anti-inflammatory compounds.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES