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Original Research Article

Evaluation of Antidiabetic Potential of Extract of *Sonneratia caseolaris* (L.) Engl. Leaves against Alloxan-Induced Diabetes in Mice

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ABSTRACT

Sonneratia caseolaris (L.), a mangrove plant of the Sundarbans have traditionally been used as an antidiabetic agent. The study investigated the potential antioxidant and antidiabetic activities of the ethanol extract of the plant leaves. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and reducing power assay. The total secondary metabolites of the plant such as the total phenolic content (TPC), total tannin content (TTC) and total flavonoid content (TFC) were also evaluated. The antidiabetic potential was evaluated using the *in vitro* alpha-amylase enzyme inhibitory assay and the *in vivo* oral glucose tolerance test (OGTT). Furthermore, Swiss albino mice were used to evaluate the effect of extract on blood glucose level of alloxan-induced diabetic mice. The extract showed the presence of a significant amount of phenolics (219.53 mg GAE/g), and flavonoids (454.88 mg QE/g). In the DPPH assay, the IC_{50} value of *S. caseolaris* was 27.0 µg/mL, while that of ascorbic acid was 12.0 µg/mL. Reducing power assay also revealed the antioxidative potential of the extract with an RC_{50} value of 395.5 µg/mL. The extract showed notable alpha-amylase inhibitory activity with an IC_{50} value of 37.6 µg/mL. In the OGTT test, extract showed a significant ($p<0.05$) reduction of blood glucose levels compared to control. Moreover, extract of *S. caseolaris* inhibited the diabetogenic activity of alloxan and significantly ($p<0.05$) controlled the blood glucose level in treated mice. The result indicated the antidiabetic potential of ethanol extract of *S. caseolaris* leaves, and therefore, justifies their use in folklore medicine.

Keywords: *Sonneratia caseolaris*, Antioxidant, Alpha amylase, Antidiabetic.

Introduction

Oxidative stress results from the increased formation of reactive oxygen species (ROS), which leads to the disintegration of the cell membrane and damages the deoxyribose nucleic acid (DNA) proteins and lipids. ROS further start or propagate the development of various chronic degenerative ailments.[1,2] The imbalance between the formation of ROS and antioxidant protection mechanism of the human body cause cellular dysfunction and plays a significant role in the development of various diseases like diabetes.[3,4] Diabetes Mellitus (DM) is a chronic metabolic complication that is characterized by hyperglycemia caused by the inhibition of insulin secretion by pancreatic β-cells and insulin resistant, leading to postprandial hyperglycemia.[5] It has been reported that the world's prevalence of diabetes is rising each year, thereby creating a major problem to the health sector, mainly in the developing countries with lack of resources. Globally, the incidence of diabetes has more than doubled during the past two decades, with over 415 million type-2 diabetic patients by the year 2015.[6] Microvascular and macrovascular complications of diabetes including the complications of heart, kidneys, eyes as well as the inhibition of antioxidant protection mechanism have extensively been observed in chronic hyperglycemic patients.[7] Routine exercises, healthy diet, and lifestyle changes are well acknowledged in controlling the hyperglycemia in patients with DM. Moreover, to inhibit the DM induced by oxidative stress, the use of antioxidant-rich natural foods or dietary supplements might also be effective.[8] Multiple drugs are available in the market for the treatment of chronic hyperglycemia. However, chronic administration of conventional antidiabetic drugs may induce serious adverse effects like cardiac failure, anemia, lactic acidosis and severe hypoglycemia in patients.[9] Various traditional medicinal systems used in many countries like North Africa, China, Sri Lanka, India, and Bangladesh use decoctions or fresh mixtures of juices prepared from medicinal plants to treat ailments like arthritis, cancer, and diabetics.[10] Consequently, many researchers are interested to find safe and effective natural antidiabetic agents from these folklore therapies for the treatment of diabetes as well as to explore their antidiabetic mechanisms.[11] *Sonneratia caseolaris* Linn. is a small evergreen plant (2 to 20 m high) with elliptic-oblong leaves (5 to 9.5 cm long), that belongs to the family Sonneratiaceae, and locally known as Ora, Choila in Bangladesh. This species is widely distributed in the tidal creek and mangrove swamps of Bangladesh, India, Sri Lanka, China, Indonesia, Malaysia, Thailand, Viet Nam, Northeast Australia, New Caledonia, and the Maldives.[12,13] About twenty-four compounds have been isolated and identified from the stems and twigs of *S. caseolaris* which includes three flavonoids, four benzene carboxylic acid derivatives, eight steroids and nine triterpenoids.[14] Some polyphenols

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like gallic acid, flavonoids like luteolin and luteolin-7-*O*-glucoside, alkaloid, carbohydrate, saponin, flavonoid, tannin and phytosterol have also been reported in this plant.[14,15] Traditionally, it is used in several countries to treat sepsis, coughs, small pox, sprains, haematuria, arresting hemorrhage, arresting bleeding and piles.[13,16] *S. caseolaris* has also been active against mosquito larvae.[17] Fruits extract of *S. caseolaris* has been reported for it hypoglycemic activity and α-glucosidase inhibitory property.[18] Based on the traditional uses, the study evaluated the potential *in vitro* antioxidative and *in vivo* antidiabetic effect of ethanol extract of the leaves of *S. caseolaris* (SCEE).

Materials and Methods

Chemicals and reagents

Ascorbic acid, acetone, *n*-hexane, chloroform, methanol, ethanol, gallic acid, quercetin, sodium nitrous, aluminum chloride, sodium hydroxide, sodium carbonate, sodium monobasic phosphate, sodium trichloroacetic acid, potassium ferricyanide, ferric chloride, dibasic phosphate, gallic acid, Iodine, hydrochloric acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Mark, Germany. Folin Ciocalteu reagent, alloxan, alpha-amylase were obtained from Sigma Chemical Co. Ltd., St. Louis, MO, USA. Glibenclamide was purchased from Incepta Pharmaceuticals Ltd., Bangladesh. Acarbose was obtained from Pacific Pharmaceuticals Ltd., Bangladesh.

Collection and authentication of plant

The leaves of *Sonneratia caseolaris* were collected in March, 2019 from Karamjol, Sundarbans, Khulna. The plant was identified by Dr. Mohammad Sayedur Rahman, senior scientific officer of Bangladesh National Herbarium (BNH), Mirpur, Dhaka-1216 and a voucher specimen was submitted to BNH (Voucher No.: 431021).

Cold extraction

The leaves were shade dried for two weeks. The dried leaves were ground into coarse powder in Wuhu motor factory, China. Cold extraction was performed to obtain the crude extract. Briefly, powdered plant material (300 g) was macerated with 90% ethanol for 10 days with occasional stirring and shaking. The mixture was filtered by Whatman filter paper and the solvent was evaporated using rotary evaporator at 50℃ to obtain crude extract. The crude extract was stored at 4℃ until further use.

Experimental animals

Female Swiss mice (4-5 weeks, weight 18-25 g) were purchased from central animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. They were kept at standard temperature (25 ± 1°C), relative humidity (56%-60%) and maintain at 12:12 (light: dark) cycle while allowing free access to food and water. All experiments were conducted according to the guidelines of Animal Ethics Committee, Ranada Prahad Shaha University (Ethical Review Certificate No.: RPSU/Registrar/ECR/Phr/2020/46).

Assessment of total content of secondary metabolites

Determination of total phenolic content

The total phenolic content (TPC) of the extract was determined spectrophotometrically using Folin-Ciocalteu (FC) reagent.[19] Gallic acid was used as a standard and different concentrations were prepared (0.5–0.1 mg/mL) to make the standard calibration curve. From each concentration, 1 mL of solution was taken into volumetric flasks followed by the addition of 9 mL distilled water and FC reagent (1 mL, 10% v/v). After 5 min, 7% sodium carbonate solution (10 mL) was added to it and the volume was adjusted to 25 mL with distilled water. After 30 min of incubation, absorbance was measured at 750 nm against a blank solution. TPC of plant extract was determined as mg gallic acid equivalent per gram of dry extract by using the calibration curve.

Determination of total flavonoid content

The total flavonoid content (TFC) of SCEE was evaluated by using standard quercetin calibration curve.[20] Various concentrations (0.5–0.1 mg/mL) of quercetin were prepared to make standard calibration curve. From quercetin concentrations, 1 mL of solution was taken into volumetric flasks followed by addition of 4 mL of distilled water and 0.3 mL of sodium nitrous solution (5% w/v). After 5 min of incubation, 0.3 mL of aluminium chloride (10% w/v) was added. Next, 2 mL of sodium hydroxide (1 M) was added and final volume was adjusted to 10 mL. Then, absorbance was read at 510 nm against a blank. TFC of SCEE was expressed as mg quercetin equivalent per gram of dry extract.

Determination of total tannin content

Ethanol leaves extract of *S. caseolaris* was investigated for total tannin content (TTC) with Folin-Ciocalteu's reagent.[20] In order to determine TTC, 10 mg of extract was dissolved in 10 mL of distilled water to prepare 1 mg/mL concentration. Gallic acid of several concentrations (500 to 15.62 µg/mL) was prepared to construct the standard calibration curve. Briefly, distilled water (7.5 mL) was mixed with 0.5 mL of Folin-ciocalteu's reagent and then 0.1 mL of ethanol extract was added. Then 1 mL sodium carbonate solution (35%) was added to the mixture and volume was adjusted to 10 mL. After incubation (30 min), absorbance was measured at 725 nm against blank. TTC of SCEE was estimated as mg gallic acid equivalent per g of dry plant extract.

Assessment of in vitro antioxidant activity

The antioxidant property of ethanol extract of *S. caseolaris* was assessed by using the qualitative and quantitative techniques. Qualitative analysis was done by Thin-layer chromatographic (TLC) technique whereas quantitative analysis was conducted by DPPH free radical-scavenging assay.

Qualitative antioxidant activity test

A suitable diluted extract solution was spotted on TLC plates. The plates were then developed in polar (chloroform, methanol and water in 40:10:1 ratio), medium polar (chloroform and methanol in 5:1 ratio) and non-polar (n-hexane and ethyl acetate in 2:1 ratio) solvent systems. After drying, the plates were sprayed with 0.05% DPPH solution in ethanol and the colour changes were observed.[20]

DPPH radical scavenging assay

DPPH free radical scavenging activity of the plant extract was measured according to the following method.[21] Plant extract was dissolved in methanol and different concentrations (1024 to 1 µg/mL) were prepared by serial dilution. 1 mL sample from each concentration was taken into test tubes and 3 mL of DPPH ethanol solution (0.004% w/v) was added to each tube. After 30 min incubation at room temperature, the absorbance was recorded at 517 nm using UV-Visible spectrophotometer (Shimadju, Japan). Ascorbic acid was used as a standard.

Percent inhibition was calculated using the following formula:

$$\%\ \text{inhibition of DPPH} = 1 - \frac{A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of blank and A_1 is the absorbance of sample or standard.

Reducing power assay

Reducing power capacity of SCEE was investigated according to the method described by Debnath *et al*.[4] Sample of different concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 mg/mL were made by serial dilution. From each concentration, 1 mL of aliquot was mixed with 2.5 mL of phosphate buffer (200 mmol/L, pH 6.6) along with 2.5 mL of potassium ferricyanide (1%) with regular shaking. After 20 min incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and then the mixture was centrifuged at 3000 rpm for 10 min. After that, 2.5 mL supernatant was collected and

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mixed with 0.5 mL ferric chloride (0.1%) with shaking. After 5 min, absorbance was measured at 700 nm against blank solution. Ascorbic acid was used as a standard compound. The reducing ability of extract was compared with standard ascorbic acid.

In vitro α-amylase inhibitory activity assays

The *α*-amylase inhibitory activity of the ethanol extract of *S. caseolaris* was investigated by the starch-iodine test.[22] Different concentrations of extract (512 to 4 µg/mL) were prepared using phosphate buffer solution (0.02 M, pH 7.0) containing 2% DMSO. Briefly, a volume of 20 µL of α-amylase solution (2 units/mL) was mixed with 1 mL of phosphate buffer and then it was added to 1 mL of each concentration of extract solution. The mixture was incubated at 37°C for 10 min. After incubation, 200 µL of starch solution (1%) was added to each test tube and re-incubated for 1 h. Then 200 µL of 1% iodine solution (5 mM I_2 and 5 mM KI) was added to the mixture followed by the addition of 10 mL distilled water to each tube. The colour change of the mixture was noted and the absorbance was measured at 620 nm. Acarbose was used as a standard drug. Sample, substrate and *α*-amylase blank solution were prepared under the same reaction conditions.

Enzyme inhibition activity was determined by;

$$\%\ \text{inhibition of enzyme} = \frac{A - C}{B - C} \times 100$$

Where, A = absorbance of the sample, B= absorbance of blank (without α-amylase), and C = absorbance of control (without starch).

Acute toxicity test

The oral acute toxicity test of SCEE was conducted according to Organization for Economic Development (OECD) Guidelines No. 425.[23] The mice were selected randomly and divided into five groups, with six animals in each group. The mice were fasted for 12 h with only free access to water. The extract was administered orally at a single dose of 500, 1000, 2000, and 3000 mg/kg body weight. Food was supplied to animals after 2 h of administration of test sample. Clinical signs and number of deaths were observed for the first 4 h, then the next 72 h and thereafter for 7 days of test sample administration. During the experiment, the behaviour of the mice such as alertness, salivation, locomotion, convulsion, diarrhoea, tremors, as well as the activity of extract on pain response, righting reflex and grip strength were observed. Additionally, mice body weight was measured on 1, 3, 5, and 7 days.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) is a standard procedure that addresses how quickly exogenous glucose can be cleared from the blood.[5] Before the experiment commenced, the mice were fasted for at least 10 h but not more than 16 h. The animals were divided into four groups i.e., control, positive control, and two test samples with six animals in each group. The positive control group was treated with glibenclamide (5 mg/kg) and the control group was administered with 1% Tween 80 in water (10 mg/kg) whereas test groups received extract (250 and 500 mg/kg). 30 minutes after the administration of standard and extracts, the mice received glucose solution orally at a dose of 2 g/kg. Blood was collected from the tail vein of each mouse by pricking with a sterile needle and the glucose level was estimated using a glucometer. Fasting blood glucose level (mmol/L) as well as blood glucose level of each mouse at 0, 30, 60, 90, and 120 min was measured. The antihyperglycemic activity of plant extract was compared with the glibenclamide and control group.[5]

Alloxan-induced antidiabetic test

The weight of overnight fasted animals with access to water were first recorded. Then the animals received a single intraperitoneal (IP) dose of freshly prepared alloxan monohydrate (150 mg/kg) to induce diabetes. The alloxan solution was prepared in normal saline (pH of 5.5). To prevent initial hypoglycemic shock, glucose solution was orally administered to the experimental mice. After 72 h of injection, blood samples were collected from the tail vein and fasting blood glucose levels of mice were recorded using an electronic glucometer. Mice having a blood glucose level of more than 10 mmol/L was marked as diabetic mice and considered for the experiment. The diabetic mice were then divided into five groups with six mice in each group. Control (normal) and diabetic control group were treated with normal saline (NaCl 0.9%) orally. Positive control group received glibenclamide (5 mg/kg) whereas mice of test groups were treated with extract (at 250 and 500 mg/kg). All administrations were done orally once a day for 21 consecutive days. Blood glucose level (mmol/L) of all mice was measured on the 3, 10, 17 and 24th day. Body weight of the mice was also recorded.[24]

Statistical analysis

The results are expressed as mean ± standard deviation (SD). Dunnett's test was performed to calculate statistical significance by one-way analysis of variance (ANOVA). Pair wise comparisons among different treatment groups were done with Post-hoc Tukey test. SPSS software of IBM Corporation, New York, USA (version 16.0) was used for analyzing the data. $P < 0.05$ was considered statistically significant.[25]

Results and Discussion

The percentage yield of *S. caseolaris* ethanol extract (SCEE) was 6.2%. The preliminary phytochemical analysis of SCEE showed the presence of reducing sugars, flavonoids, tannins, alkaloids, saponins, phytosterols, glycosides, phenolic compounds, steroids, terpenoids whereas acidic compounds and gums were absent. Many drugs that are currently being used to treat diabetes and oxidative stress appear to have notable side effects like cardiac failure, anemia, lactic acidosis and severe hypoglycemia in patients.[9] The daily uptake of natural antioxidants from plants, dietary supplements or foods, which is also an effective way of scavenging free radical, inhibits the ROS formation and prevents many diseases including DM.[8] Traditionally, *S. caseolaris* is used to treat various diseases in many countries, but local people near the Sundarbans use it as an antidiabetic.[26] Hence in our investigation, we evaluated the potential antioxidant and antidiabetic effect of SCEE.

Plant secondary metabolites are very important antioxidants and widely distributed among plant species. The total phenolic content of SCEE was found to be 219.53 mg GAE/g of dry extract. The total flavonoid and the total tannin content of SCEE was found to be 454.88 mg QE/g and 38.52 mg GAE/g of dry extract, respectively (Figure 1). Phenolics or polyphenols, known as plant secondary metabolites, are widely found in plants and are capable of producing high antioxidant activities.[27] Daily ingestion of antioxidant compounds can contribute to the development of a healthy immune system, thus protecting our body from the deleterious effect of free radicals.[8,28] Phytochemical evaluation of *S. caseolaris* showed that it contains flavonoids, tannins, alkaloids, steroids, and terpenoids. Results showed that a significant amount of total phenolics, flavonoids and moderate amount of total tannins are present in the extract (Figure 1), clearly suggesting that the plant extract is a rich source of antioxidants.

The presence of antioxidative components was demonstrated by the light yellow spots on TLC. As shown in figure 2, the extract revealed a notable DPPH radical scavenging activity and the IC_{50} value of the ethanol extract of *S. caseolaris* was 27.0 µg/mL, while the IC_{50} value of ascorbic acid was 12.0 µg/mL. In reducing power assay, the extract of SCEE exhibited a concentration-dependent moderate antioxidant activity in comparison to ascorbic acid, with an RC_{50} value of 395.5 µg/mL and for ascorbic acid it was 100.6 µg/mL (Figure 2). DPPH is a stable free radical; upon reaction with antioxidant compounds it changes the reaction colour from deep violet to light yellow.[20] The extract showed a notable free radical scavenging activity in the DPPH assay, indicating its significant antioxidant property. Compounds having reducing power are considered as a good indicator of antioxidant potential. In the reducing power assay, antioxidants decrease the potassium ferricyanide (Fe^{3+}) into potassium ferrocyanide

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(Fe^{2+}) and the addition of ferric chloride ($FeCl_3$) leads to the development of Prussian blue appearances and absorbance at 700 nm.[29] SCEE exhibited substantial reducing power activity that is comparable with ascorbic acid (Figure 2). The presence of reductones in extract showed antioxidant activity either by breaking the free radical chain or donating a hydrogen atom.[30]

The results further showed that extract and acarbose substantially inhibited the *α*-amylase enzyme activity in the starch-iodine method of *α*-amylase enzyme inhibitory activity test. The IC_{50} value of ethanol extract of *S. caseolaris* was 37.6 µg/mL whereas, for acarbose, it was 16.0 µg/mL (Figure 3). It has been reported that plant secondary metabolites like phenolic compounds, flavonoids, and tannins with free radical scavenging activity, are able to terminate, prevent or delay the oxidation of many biomolecules and weaken oxidative stress which plays a major role in managing ailments like diabetes.[31,32] The study demonstrated that SCEE exhibited high content of phenolics, flavonoids and also revealed a good DPPH free radical scavenging activity along with reducing power ability which in turn indicates its potent antioxidant property.

Several therapeutic ways to reduce the postprandial hyperglycemia like inhibition of *α*-amylase enzyme or starch blockers, can contribute to the inhibition or delay of absorption of starch through the prohibition of hydrolysis of 1,4-glycosidic bonds that converts starch to maltotriose, maltose, or other simple sugars.[33] Although, currently used oral antidiabetic drugs have high risk of hypoglycemia, lower efficacy by a long period of administration, and most importantly, failure to improve the diabetic related complications.[34] Management of diabetes without side effects is a big challenge; therefore, the World Health Organization (WHO) has suggested continuing research using traditional medicines for the remedy and management of this metabolic disorder.[35] In the *α*-amylase enzyme inhibitory property of ethanol extract of *S. caseolaris* leaves, reaction mixture having dark blue colour indicated the presence of starch and the formation of yellow colour indicated the absence of starch, while a brownish colour expressed the degradation of starch by enzyme in the mixture.[36] Results demonstrated that the extract produced dark blue colour and the measured IC_{50} value was comparable to that of acarbose (Figure 3). It has been reported that various phenolic compounds like quercetin, rutin, catechin, and procyanidins acts as *α*-amylase inhibitor by binding with proteins.[36,37] Thus, the *α*-amylase inhibition activity of SCEE is most likely due to the presence of polar compound like polyphenols, flavonoids, their glycosides, etc.

The oral administration of a single dose of SCEE (500, 1000, 2000, and 3000 mg/kg) caused no mortality in the acute toxicity test. During 7 days of observations, no signs of toxicity were noticed in the animals. The study revealed that even at a higher dose (3000 mg/kg.) the extract did not change the body weight (Table 1) and caused no mortality until the end of the experiment, this therefore, indicated that the extract is safe. The oral lethal dose (LD_{50}) of the extract was considered greater than 3000 mg/kg.

Diabetes, a metabolic abnormality, is characterized by increased blood glucose level due to lack of insulin secretion and action.[38] To observe antihyperglycemic activity by SCEE, an oral glucose tolerance test and an alloxan-induced antidiabetic test were performed. OGTT results indicated that ethanol extract of *S. caseolaris* have significant ($p < 0.05$) antihyperglycemic activity at 60, 90, 120 min compared to control (Figure 4). SCEE at a dose of 500 mg/kg showed strong antihyperglycemic activity at 90 min and the blood glucose level reduced more than 1.5 times at 120 min after glucose administration compared to the control mice. Extract at a lower dose (250 mg/kg,) also exhibited a significant reduction of glucose-induced hyperglycemia at 90 and 120 min. In addition, glibenclamide significantly ($p < 0.05$) reduced the high blood glucose level compared to control group (Figure 4).

The effect of extract on alloxan-induced diabetic mice is presented in table 2. Mice treated with alloxan demonstrated three times elevation of blood glucose level compared to the untreated mice, thereby indicating alloxan-induced experimental diabetes. The extract showed continuous lowering of the elevated blood glucose level on 17^{th} and 24^{th} day after administration of test samples compared to the diabetic control group. At 250 mg/kg, SCEE significantly ($P < 0.001$) reduced the blood glucose level (blood glucose level of diabetic control and extract at 250 mg/kg was 18.57 ± 3.4 and 10.32 ± 3.94 mmol/L, respectively at the 24^{th} day). Extract at higher dose showed marked reduction of glucose level from 15.38 ± 2.71 mmol/L to 8.07 ± 2.95 mmol/L at 3^{th} to 24^{th} day, respectively. On the 24^{th} day, SCEE (500 mg/kg dose) reduced the blood glucose level more than 2 times (glucose level for diabetic control and extract at 500 mg/kg, were 18.57 ± 3.4 and 8.07 ± 2.95 mmol/L, respectively). Glibenclamide showed a significant ($p < 0.001$) decrease in blood glucose level while comparing with untreated diabetic control mice (from 14.7 ± 3.06 mmol/L to 8.05 ± 1.16 mmol/L at 3^{th} to 24^{th} day, respectively).

Table 1: Effect of *Sonneratia caseolaris* leaf extract on body weight (g) of mice in the acute toxicity test

Dose	Day 1	Day 3	Day 5	Day 7
Control	21.03 ± 1.66	22.58 ± 2.14	24.3 ± 1.6	25.35 ± 1.75
500 mg/kg	20.52 ± 2.18 $^{\Delta}$	23.96 ± 1.58	24.5 ± 1.56 $^{\Delta}$	24.77 ± 1.13
1000 mg/kg	22.78 ± 1.53	23.01 ± 0.9	23.75 ± 1.67 $^{\Delta}$	24.52 ± 1.02 $^{\Delta}$
2000 mg/kg	22.52 ± 1.13	24.28 ± 1.38	26.1 ± 0.74	26.52 ± 2.36
3000 mg/kg	23.3 ± 0.85 $^{\theta}$	24.56 ± 1.0	27.08 ± 1.29 *$^{\theta\blacksquare}$	27.34 ± 0.89 $^{\blacksquare}$

Data are means of 6 replicates ± SD (Standard deviation); * $p < 0.05$ vs. Control (Dunnett's t test); $^{\theta}$ $p < 0.05$ vs. 500 mg/kg; $^{\blacksquare}$ $p < 0.05$ vs 1000 mg/kg; $^{\blacktriangle}$ $p < 0.05$ vs 2000 mg; $^{\Delta}$ $p < 0.05$ vs. 3000 mg/kg (pair-wise comparison by Post-hoc Tukey test).

Table 2: Effect of *Sonneratia caseolaris* leaf extract on blood glucose level (mmol/L) of mice in the alloxan-induced antidiabetic test

Dose	Day 3	Day 10	Day 17	Day 24
Control	4.45 ± 1.04	5.42 ± 1.09	5.48 ± 1.15	5.57 ± 1.53
Diabetic Control	15.93 ± 2.59 *	17.25 ± 2.73 *	18.2 ± 2.32 *$^{\blacksquare\blacktriangle}$	18.57 ± 3.4 *$^{\blacksquare\blacktriangle}$
Glibenclamide (5 mg/kg)	14.7 ± 3.06 *	14.0 ± 2.85 *	10.08 ± 3.52 $^{\theta}$	8.05 ± 1.16 $^{\theta}$
S. caseolaris (250 mg/kg)	15.68 ± 1.98 *	15.03 ± 2.72 *	12.08 ± 3.1 *$^{\theta}$	10.32 ± 3.94 $^{\theta}$
S. caseolaris (500 mg/kg)	15.38 ± 2.71 *	14.9 ± 2.63 *	10.75 ± 3.04 *$^{\theta}$	8.07 ± 2.95 $^{\theta}$

Data are means of 6 replicates ± SD (Standard deviation); * $p < 0.05$ vs. Control (Dunnett's t test); $^{\theta}$ $p < 0.05$ vs. Glibenclamide (5 mg/kg); $^{\blacksquare}$ $p <$ (*S. caseolaris* 250 mg/kg); $^{\blacktriangle}$ $p < 0.05$ vs *S. caseolaris* (500 mg/kg); pair-wise comparison by Post-hoc Tukey test.

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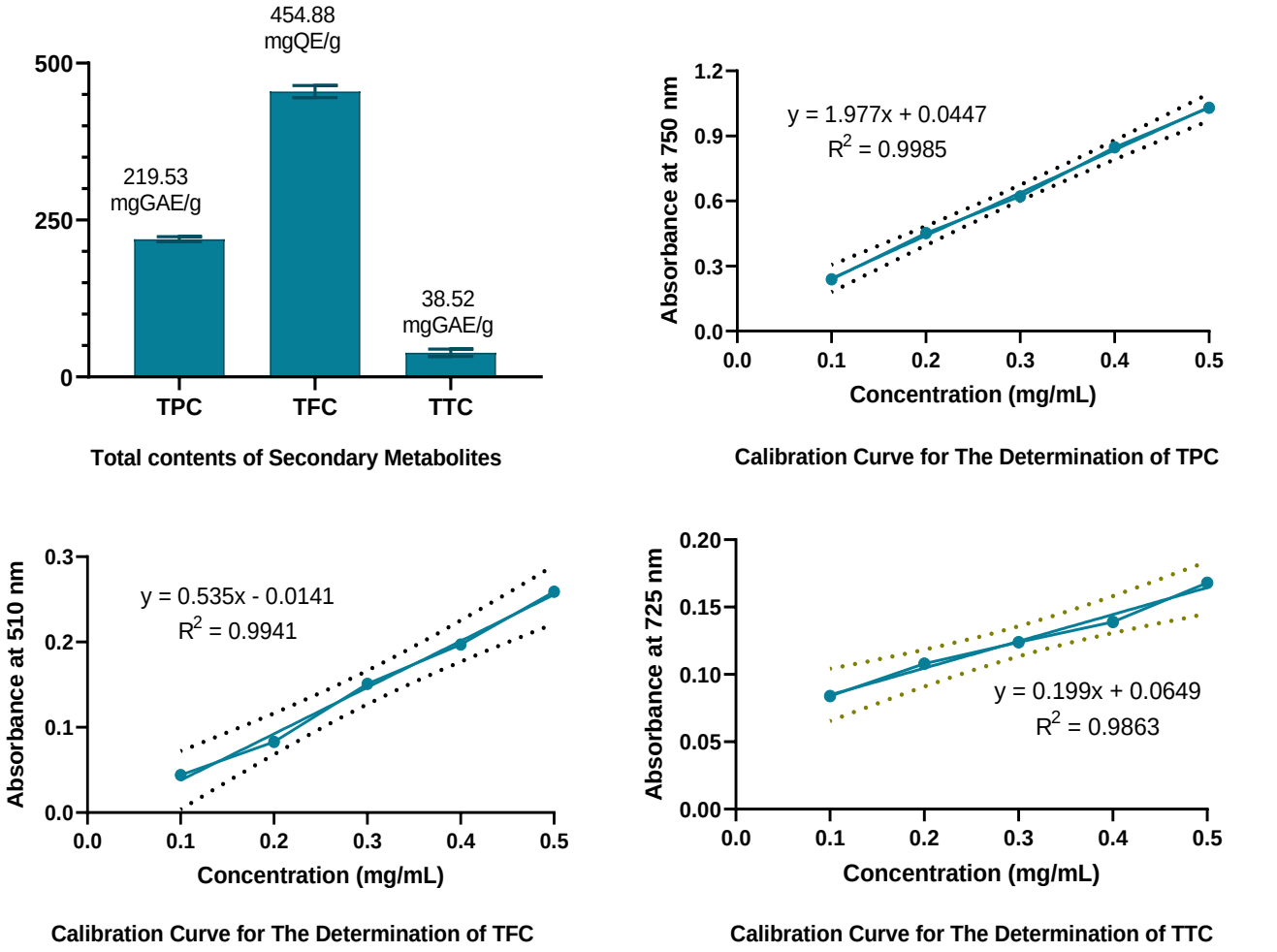

Figure 1: Determination of total phenolic content (TPC), total flavonoid content (TFC) and total tannin content of SCEE

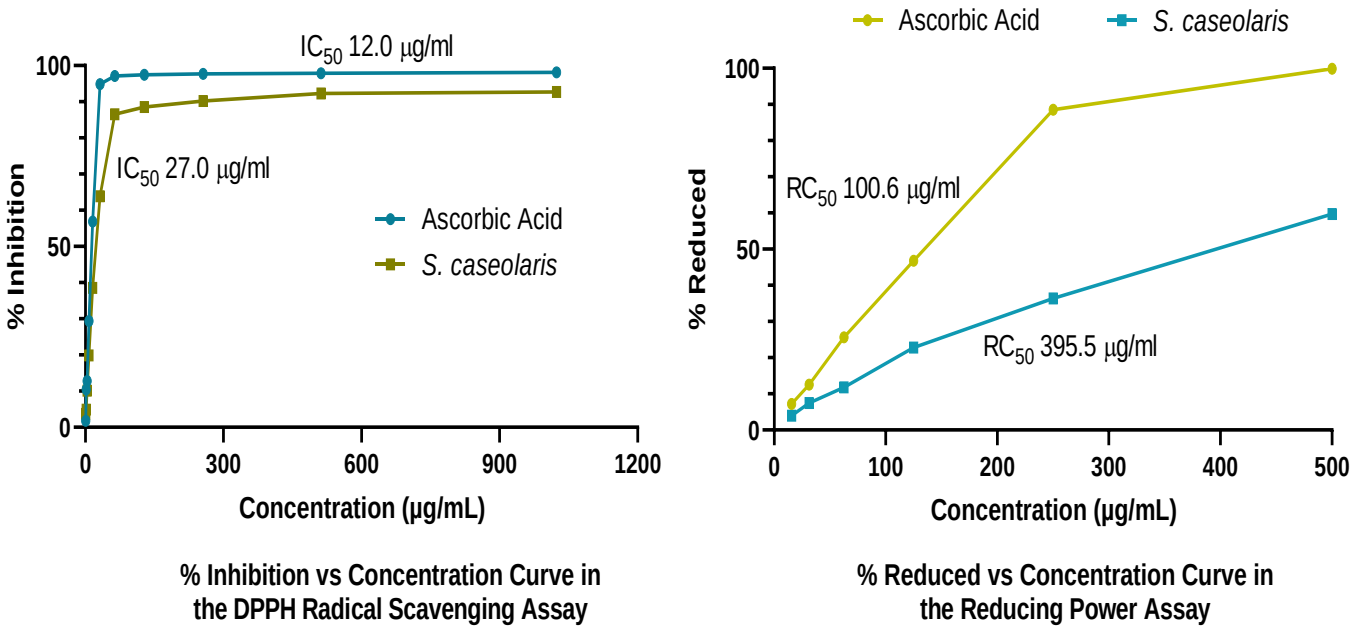

Figure 2: IC_{50} and RC_{50} values of different scavenging assays of sample extracts.

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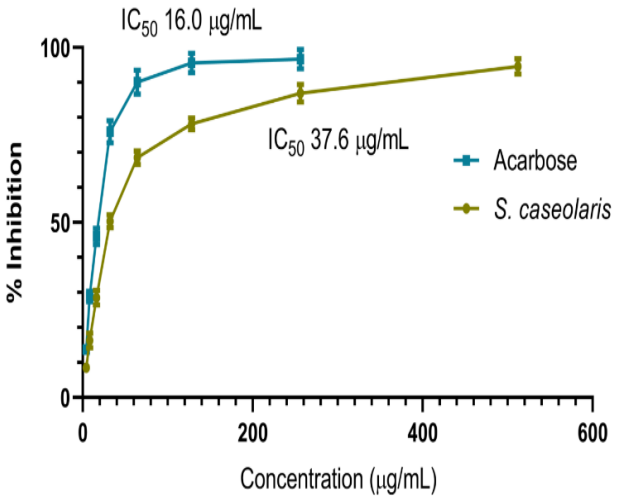

Figure 3: Determination of α-amylase enzyme inhibitory activity and the IC_{50} value of SCEE and standard drug acarbose.

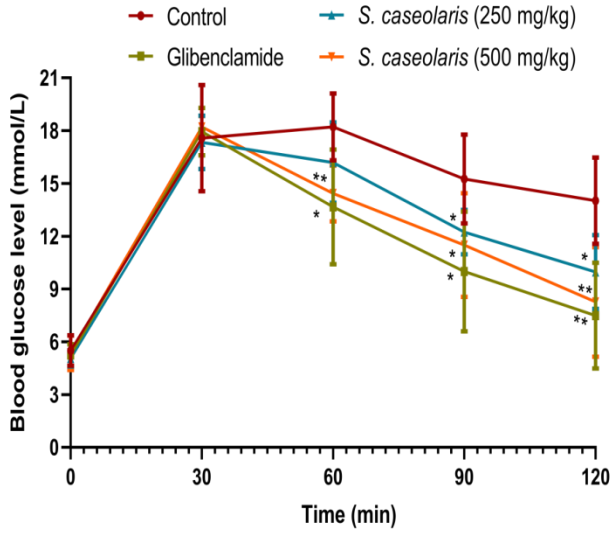

Figure 4: Effect of SCEE and standard drug on the blood glucose level of mice in the oral glucose tolerance test. Data are presented as mean ± SD; n= 6 mice/group. *P<0.05, **P<0.01 significant compared to diabetic control.

Alloxan is a toxic organic compound widely used in experiments to induce diabetes. It creates hyperglycemia by inducing the formation of free radicals, hence resulting in the destruction of pancreatic β-cells of mice and a subsequent poor insulin secretion.[39] In alloxan-induced antidiabetic test, the change between early and final blood glucose level of SCEE treated animals indicated the inhibitory action of alloxan-induced diabetogenic effect and showed good antihyperglycemic activity throughout the experiment. The significant antidiabetic activity was observed on 17th and 24th day (Table 2), clearly indicating the ability of the extract to control the elevated blood glucose level. The results suggested that, SCEE increased the glucose consumption/metabolism or improved the β-cells function, possibly due to the presence of antioxidant compounds that inhibits the production of free radical. The phenolic compounds, flavonoids, tannins of the plant may exhibit the antidiabetic activity and, flavonoids may enhance the production of the β-cells of pancreas in alloxan-induced diabetic animals.[40-42] Furthermore, it has been reported that phenolic compounds have been involved in protecting alloxan-induced toxicity and in reducing oxidative stress which raise insulin sensitivity in diabetic animals.[20,43] Thus, the marked antidiabetic activity of *S. caseolaris* leaves is attributed to the inhibition of α-amylase enzyme activity as well as free radical formation

Conclusion

Taking the results together, it may be concluded that the extract of *S. caseolaris* showed potent *in vitro* antioxidative and *in vivo* antihyperglycemic activity in alloxan-induced diabetic mice, which justifies its usage as an antidiabetic agent in folklore medicine.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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