

ORIGINAL CONTRIBUTION

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Toxicological screening and evaluation of anti-allergic and anti-hyperglycemic potential of *Sonneratia caseolaris* (L.) Engl. fruits

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Abstract

Background: *Sonneratia caseolaris* (L.) Engl. (*S. caseolaris*) belonging to the Sonneratiaceae family is commonly known as Ora. It is traditionally used as an astringent, antiseptic, to treat sprains, swellings, cough and in arresting hemorrhage. The ethanolic extract of *S. caseolaris* (L.) Engl. fruits was investigated in the present study for its toxicity as well as anti-allergic and anti-hyperglycemic potentials.

Methods: Major phenolic compounds were identified and quantified by HPLC. Behavioral change, body weight, mortality and different blood parameters were measured to assess the toxicological effect of the extract. Anti-allergic activity was evaluated using TDI-induced allergic model mice. Oral glucose tolerance test (OGTT) and STZ-induced diabetic mice were used to evaluate the anti-hyperglycemic activity.

Results: Crude extract contained ellagic acid, vanillic acid and myricetin (27.41, 3.06 and 7.93 mg per 100 g dry extract respectively). No major toxicity was observed in both acute and sub-acute toxicity study. Oral administration of the extract significantly ameliorated TDI-induced allergic symptoms like sneezing, scratching, swelling, redness and watery rhinorrhoea in the experimental mice. The extracts also reduced the total and differential count of leukocytes in the blood. The extract treated mice showed significant reduction in blood glucose, SGOT, SGPT, cholesterol, triglycerides, urea, creatinine and bilirubin level.

Conclusions: *S. caseolaris* contains bioactive phytoconstituents which may be the possible precursors to isolate and characterize the novel compounds targeting the diseases like allergy and diabetes.

Keywords: *Sonneratia caseolaris* (L.) Engl., Allergy, Diabetics and phenolic compounds

Introduction

Hypersensitivity or hyperactivity of immune system is known as allergy [1] which is one of the serious immune dysfunctions worldwide. Food, pollen, dust, mites, chemical, cosmetics, mold spores, animal hairs etc. cause allergic syndromes and are known as allergen [2]. Toluene

2,4-diisocyanate (TDI) is one of the leading causes of profession related allergic diseases such as allergic rhinitis, scratching, sneezing, rhinorrhea, swelling, redness and bronchial asthma [3–6]. People may be affected by TDI in their work premises due to lack of standardized protective environment or gears [7–9]. Present antihistamines like chlorpheniramine, cetirizine, loratidine, fexofenadine, rupatidine, olopatadine etc. cause different adverse effects including dry mouth, drowsiness, dizziness, headache, blurred vision and so on [10]. This

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situation has prompted researchers to investigate new antiallergic drugs [11].

Diabetes mellitus (DM), a complex, chronic endocrine disease, is occurred by inherited and/or acquired deficiency in insulin production by the pancreas or by the ineffectiveness of the insulin produced. It is a major health problem in most countries and is considered to be high (4-5 %) all over the world [12]. Chronic hyperglycemia causes severe complications linked to diabetes and is a common cause of chronic morbidity and mortality around the world. In spite of the availability of many hypoglycemic agents, diabetes and its linked complications are still an important medical problem [12]. This hyperglycemic state generates classical symptoms viz. polyuria, polydipsia, polyphagia and weight loss [13]. The deficiency of β -cells of the endocrine pancreas and subsensitivity to insulin in target cells cause the high concentration of blood glucose and other biochemical abnormalities [14, 15].

Plants are traditionally used for the treatment of diabetes since ancient times and acted as an exemplary source of medicine [16]. The treatment and management of DM has been considered to use of oral hypoglycemic agents and insulin. But these drugs have some characteristic serious side effects [17] including loss in body weight, polyphagia, polyuria and polydipsia and these increased catabolic reactions leading to muscle wasting might be the cause of the reduced weight [18]. This leads to increasing demand for herbal products with effective anti-diabetic potential with little side effects and relatively low cost.

The plants show anti-allergic and anti-hyperglycemic activity due to their ability to restore different secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids and carotenoids, polyphenols [19].

HPLC-DAD procedure provided excellent identification and quantification of phenolic compounds in the ethanolic extract of *S. caseolaris* fruits within a short analysis time (40 min). The plants rich with polyphenols can act as antioxidants, anti-inflammatory [20] and anti-diabetic [21].

S. caseolaris is an evergreen tree usually up to 15 m tall and has dark red-petalled flowers and its green fruits are round, leathery berries that are up to 7.5 cm wide [22]. It is a true mangrove species abundantly found in Bangladesh, and Asian tropics [23]. Traditionally fruits are used to treat bleeding, hemorrhages, piles, sprain and poultices [24]. Extracts of this plant are also traditionally used as an astringent and antiseptic [25]. It is a folk remedy for worms [26]. Half-ripe fruits are a treatment for coughs [26]. Leaf is used against diarrhea [24]. This plant has been reported to have antioxidant [27], antimicrobial [28], antifungal activity against *F. oxysporum* and leaf as a potential source for antidiabetic

agents [29]. Scientific investigations claimed that the plant contains a wide range of compounds including luteolin and luteolin 7-*O*- β -glucoside [30], oleanolic acid, β -sistosterol- β -d-glucopyranoside [31]. Due to the presence of lots of valuable constituents, the presents study was undertaken to investigate the anti-allergic and anti-hyperglycemic activity of *S. caseolaris* fruit extracts. Acute and sub-acute toxicity study are short term assessment and evaluation of potential hazards of test substance. That's why toxicological study was also carried out for *S. caseolaris* fruits.

Materials and methods

Chemicals and reagents

Toluene 2,4-diisocyanate (TDI) was purchased from Wako Chemical, Tokyo, Japan. Streptozotocin (STZ) was purchased from Sigma-Aldrich, Germany. Standard drug cetirizine and glibenclamide were collected from Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh. All other chemicals including ethyl acetate, acetic acid were of analytical grade.

Preparation of plant extracts

For the present investigation, the fruits of *S. caseolaris* was collected from Sunderbans Mangrove forest, Khulna and was identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka, where voucher specimen were submitted (DACB 43,821) for future reference. The collected fruits were separated from undesirable materials and 20 days shade dried after complete drying, the fruits were grinded into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). About 500 gm of powder was taken in clean, flat-bottomed glass containers and soaked in 1500 ml of 96 % ethanol. The container with its contents was sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of clean cloth followed by filtration through Whatman filter paper. The filtrate obtained was concentrated using rotary evaporate. It rendered a gummy concentrate of brownish yellow color (yield: 3.0 %). The gummy concentrate was designated as crude ethanolic extract of *S. caseolaris* and stored in a refrigerator (4 °C) for further use.

HPLC detection and standardization of crude extract

Standardization of crude extract with some selected pure compounds were determined by HPLC-DAD analysis as described by Sarunya et al., 2006 [32]. It was carried out on a Dionex UltiMate 3000 system equipped with quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). Separation was performed using Acclaim[®] C₁₈ (5 μ m) Dionex

column (4.6 × 250 mm) at 30 °C with a flow rate of 1 ml/min and an injection volume of 20 µl. The mobile phase was consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B), and methanol (solvent C) with the gradient elution program of 5 %A/95 %B (0–5 min), 10 %A/90 %B (6–9 min), 15 %A/75 %B/10 %C (11–15 min) [11–15], 20 %A/65 %B/15 %C (16–19 min), 30 %A/50 %B/20 %C (20–29 min), 40 %A/30 %B/30 %C (30–35 min) and 100 %A (36–40 min). The UV detector was set to 280 nm for 22.0 min, changed to 320 nm for 28.0 min, again change to 280 nm for 35 min and finally to 380 nm for 36 min and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 nm to 700 nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing arbutin (AR), (-)-epicatechin (ECA) (5 µg/ml each), gallic acid (GA), hydroquinone (HQ), vanillic acid (VA), rosmarinic acid (RA), myricetin (MC) (4 µg/ml each), caffeic acid (CA), Syringic acid (SA), vanillin (VL), *trans*-ferulic acid (FA) (3 µg/ml each), *p*-coumaric acid (PCA), quercetin (QU), kaempferol (KF) (2 µg/ml each), (+)-catechin hydrate (CH), ellagic acid (EA) (10 µg/ml each), *trans*-cinnamic acid (TCA) (1 µg/ml), rutin hydrate (RH) (6 µg/ml) and benzoic acid (BA) (8 µg/ml). The sample was mixed with 30 % ethanol; vortexed for 20 min, sonicated for 15 min and then filtered through a 0.20 micron syringe filter and run in the HPLC system. Data acquisition, peak integration, and calibrations were calculated with Dionex Chromeleon software (Version 6.80 RS 10).

Experimental animals

Young Swiss-albino mice aged 5–6 weeks, weight 22–25 gm were used for the experiment. Before their use, they were kept for one week in different cages at standard laboratory conditions (temperature 25–28 °C and 12 h light/dark cycle) in animal house of Pharmacy Discipline, Khulna University, Bangladesh for adaptation. Animals were procured from Jahangirnagar University, Bangladesh. All animal experiments were carried out following the guidelines of Animal Ethics Committee, Pharmacy Discipline, Life Science School, Khulna University, Bangladesh (KU/PHARM/AEC/15/06/026).

Toxicological screening

Acute toxicity study

Acute toxicity study was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines – 425 with slight modifications [33]. In short, male Swiss albino mice were divided into five groups denoted as control, test-I, test-II, test III and test IV consisting of six mice in

each group. Control was treated with 2 % tween 80 in water while test-I, test-II, test III and test IV group were administered the ethanolic extract of *S. caseolaris* fruits at dose of 0.5 g/kg, 1 g/kg, 2 g/kg and 3 g/kg body weight. Prior to any treatment, each mouse was weighed properly and the doses of the test sample, control material were adjusted accordingly. Animals were observed individually during the first 30 min after dosing and then at every 24 h for 14 days for any clinical sign of toxicity (behavioral) or mortality.

Sub-acute toxicity study

The sub-acute toxicity test of ethanolic extract of *S. caseolaris* (L.) Engl. fruits was performed according to the procedure previously described by Ghosh et al., [34] with slight modifications. Ten experimental animals were randomly selected and divided into two groups denoted as control and test consisting of six mice in each group. Test group was orally administered extract at 500 mg/kg daily for 14 days while the control group received vehicle only (2 % tween water solution). After extract treatments, all the experimental animals were observed daily for any abnormal clinical signs and mortality for 14 days. At the end of 14 days, the mice were anaesthetized and their blood samples was collected from the cervical vein [35] and kept in a non-heparinized tube for biochemical analysis, blood without additive was centrifuged at 3000 rpm for 10 min. Serum was separated and then SGPT, SGOT, bilirubin, urea and creatinine were estimated using a blood analyzer.

Anti-allergic activity evaluation

The test was carried out as per the procedure previously described by Dev et al., [1] and Sardar et al., [36] with slight modifications. Experimental animals were randomly selected and divided into five groups denoted as group-I, group-II, group-III, group-IV and group-V consisting of six mice in each group. Group-I (negative control) received ethyl acetate (10 µl) bilaterally on the nasal vestibules and 2 % tween 80 water orally. Group-II served as positive control and was given TDI (10 µl of 5 % TDI solution in ethyl acetate) bilaterally on the nasal vestibules and 2 % tween-80 in water equivalent to the vehicle given with the extract. Group-III served as standard and was given TDI (10 µl of 5 % TDI solution in ethyl acetate) bilaterally in the nasal vestibules and antihistamine (cetirizine, 20 mg/kg body weight) orally. Group-IV and Group-V received ethanolic extract of *S. caseolaris* fruits (300 mg/kg and 500 mg/kg body weight respectively) orally and TDI (10 µl of 5 % TDI solution in ethyl acetate) bilaterally on the nasal vestibules (Fig. 1).

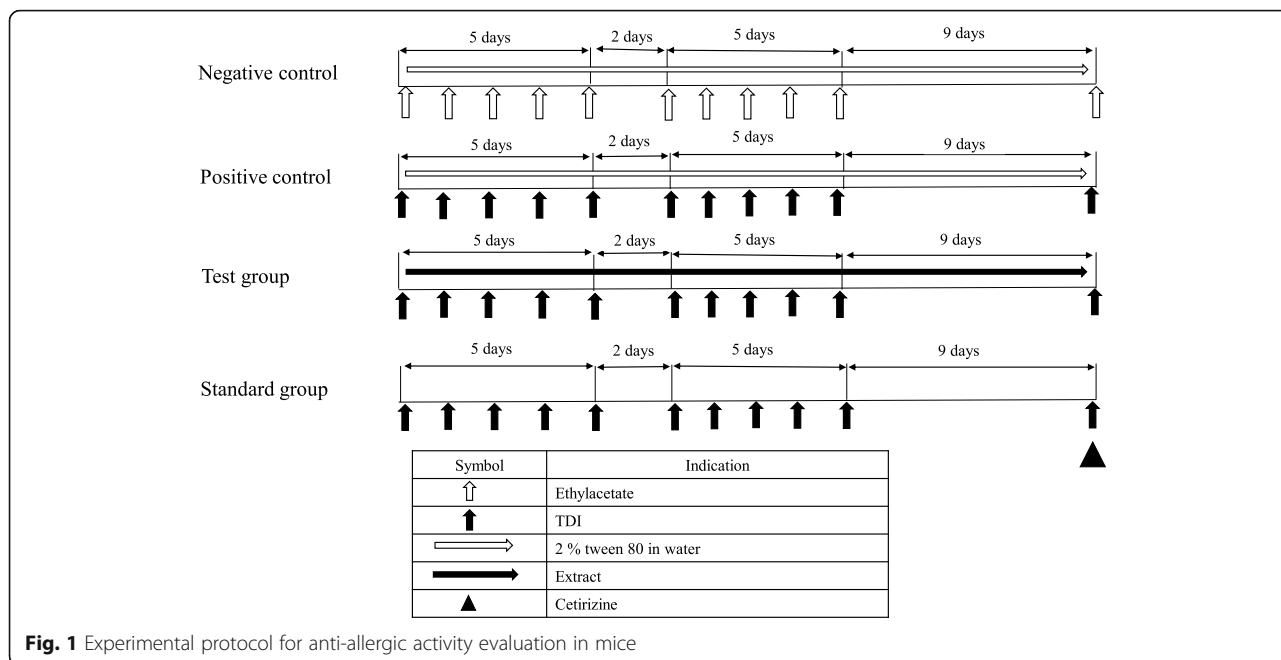


Fig. 1 Experimental protocol for anti-allergic activity evaluation in mice

Assessment of allergy-like symptoms

Nasal allergy-like symptoms were measured immediately after TDI provocation [37] and for 10 min by placing the animals in different cages. The number of sneezing, number of scratching and nasal score i.e. the extent of watery rhinorrhea, swelling, and redness were measured [38]. The nasal score was measured on a grading scale ranging from 0 to 3 (Table 1), where 0 is lowest and 3 is highest [35].

Blood sample collection and differential analysis

After observing the allergy-like symptoms, mice were anesthetized and blood was collected from the cervical vein following the method described by Mahajan et al., [35]. The collected blood was taken in a heparinized tube and used for total and differential leukocyte count. The blood was diluted at 1:10 ratio with 1 % acetic acid to lyse red blood cells and total leukocytes were counted using automated cell counter (DS-500i, 5 part automated hematology analyzer; Edan Instruments Inc., Shenzhen, China). For determining differential counts of leukocytes, slides were prepared and were then stained with Field’s stain. Following drying of the

slides, 300cells/slide were counted on a compound microscope (oil immersion power) at 400× magnification.

Anti-hyperglycemic activity evaluation

Oral Glucose Tolerance Test (OGTT)

OGTT of *S. caseolaris* extract was carried out following the method adopted by Joy et al., [39] with slight modification. The experimental animals were fasted for 12 h. The mice were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV consisting of six mice in each group. Each group received a specific treatment i.e. control (2 % tween water solution), standard (10 mg/kg Glibenclamide) and the test sample (300 mg/kg and 500 mg/kg extract) orally. After 30 min later glucose solution (2 gm/kg body weight) was administered to all groups orally. Using the glucometer (Accu-check Active, Model No.: 0665619021) blood glucose levels were measured at 0 min, 30 min, 90 and 150 min after glucose administration. The blood glucose level was expressed in mMol/L.

Table 1 Criteria for grading the severity of TDI-induced nasal responses in mice

Allergic symptoms	Nasal Score			
	0	1	2	3
Nasal response	0	1	2	3
Watery rhinorrhoea	(-)	At the nostril	Between 1 and 3	Drops of discharges
Swelling and redness	(-)	Slightly swollen	Between 1 and 3	Strong swelling with redness

Induction of diabetes by STZ

Mice were given STZ following the established methods [40, 41] with slight modification. In short, the required quantity of STZ (120 mg/kg) was dissolved in isotonic solution (0.9% NaCl) and injected intraperitoneally [42]. After 72 h, the mice with moderate diabetes having glycosuria and hyperglycemia were considered as diabetic mice and used for further experiment.

Experimental design

Mice were divided into five groups consisting of six animals in each group. Group I, control mice were administered 0.5 mL 2% tween water solution every day orally for 28 days. Group II, diabetic control mice were administered 0.5 mL 2% tween water solution every day orally for 28 days. Group III and Group IV, diabetic mice were administered with 300 mg/kg and 500 mg/kg extract per day orally for 28 days; Group V, diabetic mice was administered with 10 mg/kg glibenclamide solution orally per day for 28 days. The fasting (withdraw food before 12 h) blood glucose and body weights were measured on 7th, 14th, 21th and 28th days. At the end of the experiment, mice were sacrificed and blood was collected for biochemical studies.

Blood and urine glucose determinations

Blood samples were collected from the mice tail-tip amputation for the determination of blood glucose according to method mentioned by Togashi et al., [43].

Changes in the body weight and blood glucose level of each group were determined weekly [44]. Urine was collected weekly and glucose was checked using URIC 3VC kit [45].

Biochemical estimations

After 28 days of treatment, the mice were fasted for 16 h. The animals were then sacrificed by cervical decapitation and blood was collected in the tubes [35, 46]. Blood samples were centrifuged for 10 min at 1500 rpm to separate the serum and serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum urea, serum creatinine, serum bilirubin, serum cholesterol, and triglycerides levels were assessed by blood analyzer using commercially available kits [47].

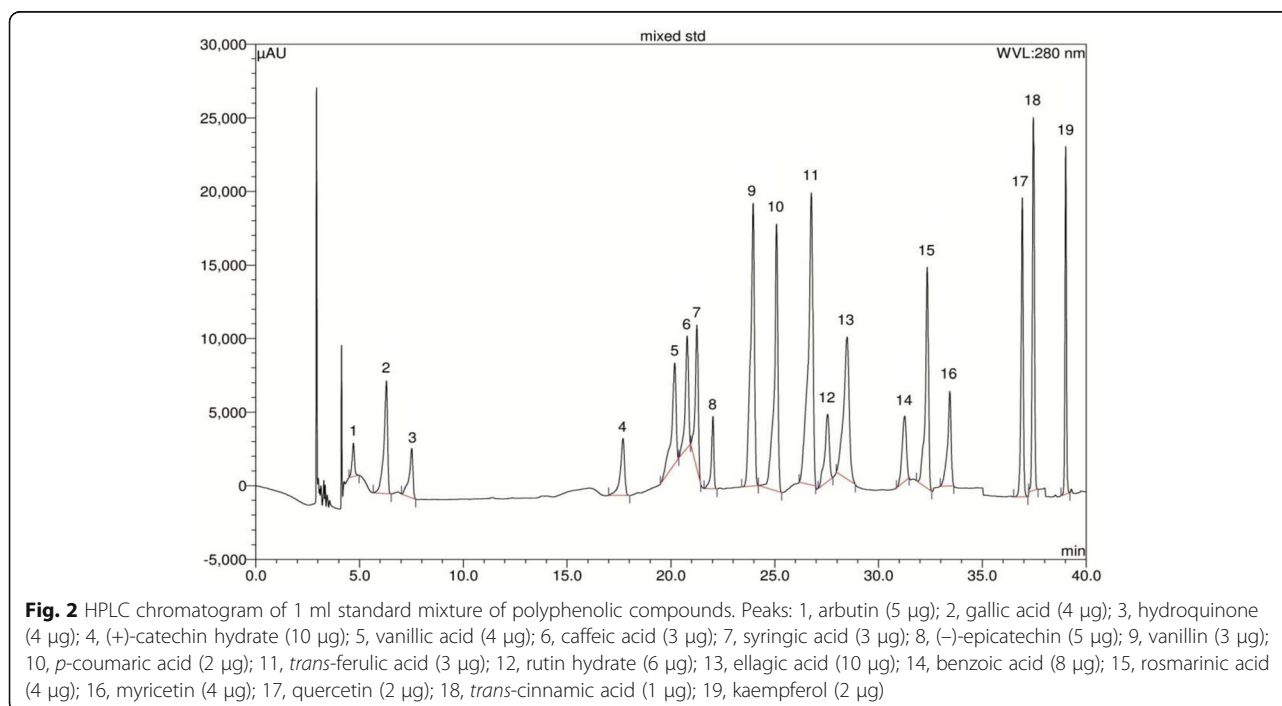
Statistical analysis

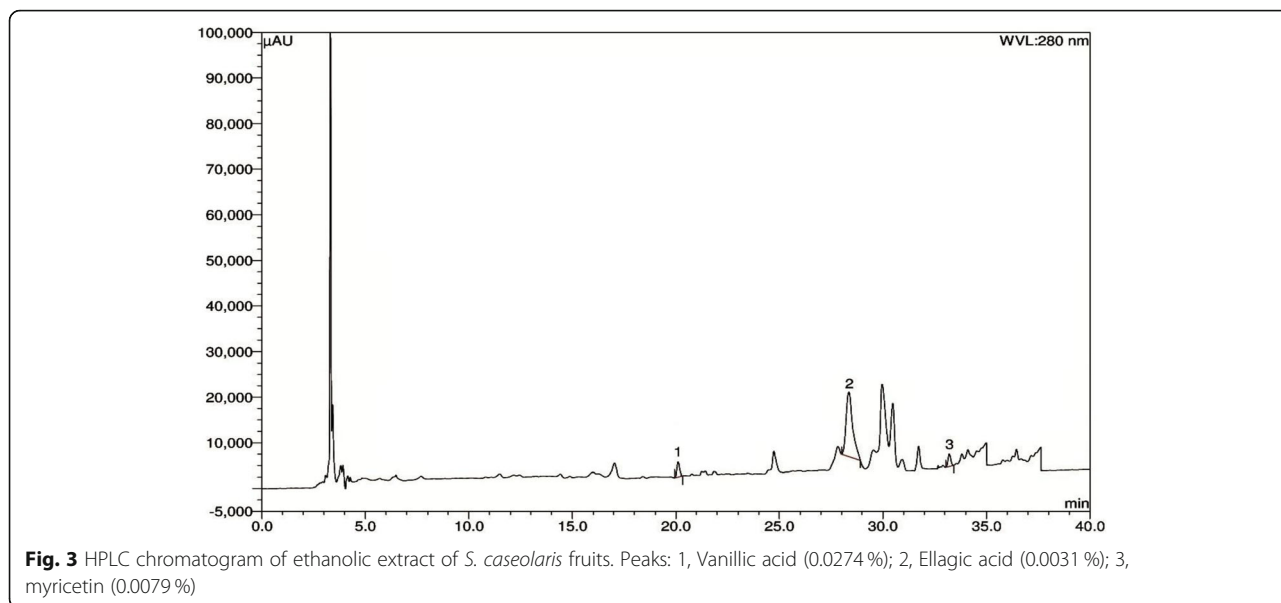
All experiments were duplicated and results were presented as mean \pm SEM. Statistical analysis of all the data obtained was evaluated using one-way ANOVA. Experimental results were considered statistically significant when $p < 0.05$.

Results

Standardization of crude extract

The individual phenolic compounds in the ethanolic extract of *S. caseolaris* fruit was standardized by HPLC. The HPLC separations of polyphenols in standard and





fruit extract were shown in Figs. 2 and 3 respectively. The content of each phenolic compound was calculated from the corresponding calibration curve and presented as the mean of five determinations. The experimental results indicated that the crude extract contained a moderate concentration of ellagic acid (27.41 mg/100 g of dry extract). Vanillic acid and myricetin were also detected at lower concentration (3.06 & 7.93 mg/100 g of dry extract, respectively). Since the phenolic compounds have been of interest against allergy and diabetes, the present HPLC study could be a potential application to identify and quantify the polyphenolic compounds in the extracts targeting allergy and diabetics.

Acute toxicity study

Acute toxicity study revealed the non-toxic nature of the extracts. The *S. caseolaris* fruit extract on tested mice proved the safety of this plant (Table 2). There was no

lethality or any toxic reactions found at any of the doses even at 3000 mg/kg until the end of the study period, suggesting the LD₅₀ of the extract of the plant is above 3000 mg/kg (Table 3).

Sub-acute toxicity study

In sub-acute toxicity study, test mice were treated with EESC at 500 mg/kg doses for 14 days orally and the effects were monitored on different biomarkers. None of the studied parameters showed any evidence of adverse effects at 500 mg/kg extract treated mice as compared to control group (Table 4).

Anti-allergic activity study

Effect on TDI-induced nasal allergy-like symptoms

Intranasal application of TDI induced nasal allergy like symptoms such as sneezing, watery rhinorrhea, redness, swelling and itching. In TDI-sensitized mice, the total

Table 2 Effect of ethanolic extract of *S. caseolaris* (L.) Engl. fruit on behavioral aspects

Observation	Control(2 % tween Solution, 0.5 mL/mice)	Test I (0.5 gm/kgw) EESC	Test II (1 gm/kbw) EESC	Test III (2gm/kbw) EESC	Test IV (3gm/kbw) EESC
Food Intake	Normal	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal	Normal
Change in Skin	No Change	No Change	No Change	No Change	No Change
Drowsiness	Not Present	Not Present	Not Present	Not Present	Not Present
Sedation	No Effect	No Effect	No Effect	No Effect	No Effect
Eye Color	No Change	No Change	No Change	No Change	No Change
Diarrhoea	Absent	Absent	Absent	Absent	Absent

Table 3 Effect of ethanolic extract of *S. caseolaris* fruits on mortality and body weight

Group	No. of treated mice	No. of dead mice	Average initial body weight (gm) ± SEM	Average bwt. after 14 days (gm) ± SEM
Control (2% tween, 0.5 mL/mice)	06	0	21.56 ± 1.22	24.83 ± 1.38
Test I (EESC, 0.5 gm/kbw)	06	0	22.67 ± 1.25	25.12 ± 1.61
Test II (EESC, 1 gm/kbw)	06	0	23.46 ± 1.96	25.82 ± 1.19
Test-III (EESC, 2 g/kbw)	06	0	23.72 ± 2.09	25.49 ± 1.33
Test-IV (EESC, 3 g/kbw)	06	0	23.11 ± 0.97	26.42 ± 1.23

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

**p* < 0.05 vs control

number of sneezes, scratches and the nasal score were 31 ± 2.78, 161.33 ± 6.73 and 3 respectively. Standard drug cetirizine significantly improved these symptoms and the values were 13.75 ± 1.49, 63.0 ± 9.93 and 0.4 ± 0.24 respectively. Oral administration of EESC also significantly suppresses the number of sneezing, scratching and the nasal score at both the doses (Table 5).

Effect on WBC

In the blood samples of the mice of TDI-control group, the total numbers of circulating leukocytes, eosinophils, lymphocytes and neutrophils were markedly increased (*p* < 0.05) in comparison with control group, although the numbers of monocytes and basophils were not significantly different. In contrast, oral administration of EESC significantly decreased the total count of WBC and the number of neutrophils, lymphocytes and eosinophils in a dose dependent manner compared with TDI control group. The efficacy of extract at a dose of 500 mg/kg was comparable with the standard antihistamine (Table 6).

Oral glucose tolerance test

Oral administration of *S. caseolaris* (L.) Engl. fruits extract to glucose-loaded mice at doses of 300 and 500 mg/kg body weight led to dose-dependent reductions in blood glucose levels compared to control mice (Table 7).

Anti-diabetic activity

In STZ-induced diabetic mice, the blood glucose levels were increased and body weight were decreased. Administration of EESC at both doses significantly lowered the blood glucose but increased the body weight in STZ-induced diabetic mice (Tables 8 and 9).

Biochemical parameters in blood

Oral administration of *S. caseolaris* resulted a significant (*p* < 0.05) reduction of SGPT, SGOT, serum triglyceride, total cholesterol, creatinine, urea and bilirubin in mice compared to the diabetic mice. The result of serum biochemical parameters in non-diabetic and diabetic condition mice were presented in Table 10. The diabetic control mice showed a significant increase in particular biochemical parameter as compared with normal control. The extract was found effective in diabetic related complications and the activity was quite promising and comparable with standard drug glibenclamide.

Glucose level in urine

Urine analysis on 7th day showed the presence of glucose (+++) in the entire group, except control group. But the continuous administration of ethanolic extract of *S. caseolaris* (L.) Engl. was found to be effective in reduction of urine glucose level in diabetic mice at the end of the experiment (Table 11).

Table 4 Effect of ethanolic extract of *S. caseolaris* fruits on different biomarkers

Parameter	Control (2% tween Solution, 0.5 mL/mice)	Test (EESC 500 mg/kbw)
Urea	25 ± 0.87 mg/dL	27 ± 0.33 mg/dL
Creatinine	1.5 ± 0.21 mg/dL	1.33 ± 0.36 mg/dL
Bilirubin	1.0 ± 0.06 mg/dL	1.25 ± 0.09* mg/dL
SGPT (ALT)	90 ± 1.39 U/L	70 ± 1.05* U/L
SGOT (AST)	65 ± 0.99 U/L	80 ± 1.88* U/L

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

**p* < 0.05 vs. control

Table 5 Assessment of allergy-like symptoms in mice

Group	No. of sneezing	No. of Scratching	Nasal score
Negative Control (ethyl acetate only)	2.33 ± 1.86	19.33 ± 6.69	0
Positive control (5 % TDI treated)	31.00 ± 2.78 [#]	161.33 ± 6.73 [#]	3.0 ± 0.00
Standard (Cetirizine, 20 mg/kg)	13.75 ± 1.49*	63.0 ± 9.93*	0.4 ± 0.24*
Test-I (EESC, 300 mg/kg)	26.43 ± 2.59*	126.71 ± 14.03*	1.2 ± 0.2*
Test-II (EESC, 500 mg/kg)	20.33 ± 2.64*	96.5 ± 10.74*	0.4 ± 0.24*

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

indicates $p < 0.05$ vs. negative control and * indicates $p < 0.05$ vs. Positive control

Discussion

The objective of the present study was to evaluate anti-allergic and anti-diabetic potential of ethanolic extract of *S. caseolaris* fruits. The acute toxicity studies revealed the nontoxic nature of this plant which was evident by the absence of mortality or any signs of behavioral abnormalities even after oral administration of extracts up to the single dose level of 3000 mg/kg body weight in mice (Tables 2 and 3). The fruit extract was also found to be safe at low dose for longer period as it was ascertained by examining some biochemical parameters such as SGPT, SGOT, bilirubin, creatinine and urea (Table 4). These ensure the safety of this extract as crude medicine.

In the study of anti-allergic potential, TDI was used to sensitize and provoke allergy-like symptoms, e.g., sneezing, scratching, rhinorrhea, redness and swelling [36]. The investigations were also carried out on total and differential count of WBC such as eosinophil, basophil, neutrophils, lymphocyte and monocytes. The ethanolic extract of *S. caseolaris* (L.) Engl. fruit significantly decreased the number of sneezing, scratching, swelling, redness and rhinorrhea (Table 5) in dose dependent manner. It has been reported that sneezing and nasal rubbing is caused by histamine through its binding to histamine H₁ receptor (H₁R) on the sensory nerve endings [48]. H₁R

plays a key role in histamine signaling involved in the allergic response [49]. Moreover swelling and rhinorrhea mediators leukotriene and prostaglandins also play major roles [49] and increase IgE levels [50]. It has been reported that TDI-induced allergic response is a neurogenic and IgE-dependent [51]. In our study we have found that the total leukocytes, eosinophils, lymphocytes and neutrophils have increased in blood of TDI-control mice but treatment with the ethanolic extract of *S. caseolaris* fruits at 300 mg/kg and 500 mg/kg significantly reduced the count of these WBC cells as compared to TDI-control (Table 6). This increased eosinophilia and leukocytes work as a good cellular biomarker for allergic symptoms [52]. The results (Table 6) obtained from the present study were consistent with previous findings and hypothesis presented by several researchers [36, 53, 54]. HPLC analysis exhibited that ethanolic extract of *S. caseolaris* fruit contain diverse phenolic compounds and bioactive metabolites especially myricetin, vanillic acid and ellagic acid (Figs. 2 and 3) which might be responsible for anti-allergic effects. Polyphenolic compounds e.g., gallic acid inhibit the release of histamine and helper T cell cytokines, IL-4 and IL-2 form mast cells in numerous experimental animal model of allergic diseases [55, 56].

Table 6 Total and differential count of WBC in blood of experimental mice

Group	× 10 ³ cells /ml					
	TC (WBC)	Lymphocyte	Neutrophil	Eosinophil	Monocyte	Basophil
Control (Ethyl acetate treated)	8.57 ± 0.46	5.57 ± 0.81	1.80 ± 0.33	1.03 ± 0.03	0.17	0.00
TDI control	14.34 ± 0.67*	8.92 ± 0.86*	5.01 ± 0.82*	1.45 ± 0.02*	0.02	0.07
Standard (Cetirizine, 20 mg/kg)	9.77 ± 0.69**	6.25 ± 0.47**	2.05 ± 0.72**	1.37 ± 0.30**	0.10	0.00
Test-I (EESC, 300 mg/kg)	8.49 ± 0.75**	4.92 ± 0.50**	2.55 ± 0.67**	0.68 ± 0.05**	0.17	0.08
Test-II (EESC, 500 mg/kg)	6.62 ± 0.87**	3.97 ± 0.62**	2.38 ± 0.23**	0.20 ± 0.01**	0.07	0.00

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

* indicates $p < 0.05$ vs. control and ** indicates $p < 0.05$ vs. TDI control

Table 7 Hypoglycemic effect of ethanolic fruit extract of *S. caseolaris* (L.) Engl

Group	Blood glucose level (mMol/L)			
	Fasting state	30 min	90min	150 min
Group-I (Control, 2% tween solution, 0.5 mL/mice)	4.44±0.56	13.54± 0.35	9.22 ± 0.56	8.02 ± 0.28
Group-II (Standard, Glibenclamide, 10 mg/kg)	4.22 ± 0.30	2.8 ±0.26*	3.18 ± 0.25*	3.74 ± 0.18*
Group-III (EESC 300 mg/kg)	3.54 ± 0.70	8.32 ± 0.97*	7.46 ± 0.35****	6.96± 0.23***
Group-IV (EESC, 500 mg/kg)	4.14 ± 0.24	6.98 ± 1.02*	5.94 ± 0.34**	5.32 ± 0.21*

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

*indicates $p < 0.0001$ compared with control

** indicates $p < 0.001$ compared with control

*** indicates $p < 0.01$ compared with control

**** indicates $p < 0.05$ compared with control

Insulin-dependent diabetes mellitus (IDDM) is a disease caused by progressive destruction of the insulin secreting β -cells [57]. Insulin is a peptide hormone that elicits the glucose metabolism and maintain the normal glucose level in blood [58]. The preliminary screening and HPLC analysis of *S. caseolaris* fruits revealed the presence of phenolic and flavonoids in considerable amounts. All these compounds have been reported to possess strong anti-diabetic potential [59, 60]. The extracts showed significant reduction on blood glucose level in different tested hours in oral glucose tolerance test (Table 7).

The increase in blood glucose level is important characteristics feature of diabetic condition. Prolonged administration (28 days) of *S. caseolaris* at both (300 mg/kg and 500 mg/kg) doses resulted in a significant reduction of blood glucose levels as compared to the STZ-induced diabetic mice (Table 8). STZ-induced diabetes is also characterized by a severe loss in body weight, polyphagia, polyuria and polydipsia [61] and these may be occurred by catabolic reactions leading to muscle wasting in diabetic mice. Oral administration of the extract moderately

improved the body weight in diabetic mice (Table 9). In these studies, the significant increase in serum SGOT and SGPT levels in STZ-induced diabetic mice represents liver damage compared to control mice. Liver necrosis in STZ-induced diabetic mice increased the presence of SGPT and SGOT and bilirubin in plasma by leakage of the enzymes from liver cytosol into the bloodstream. In extract treated mice significant decrease of these biochemical parameters were observed (Table 10).

Furthermore, the significant increase in serum creatinine and urea levels in STZ-induced diabetic mice indicates the kidney damage compared to control mice. But the oral administration of extracts showed a positive impact on the kidney function through reducing the serum concentrations of creatinine and urea (Table 10). Abnormalities in the lipid profile are one of the most common complications in DM and have an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction [62]. The elevated cholesterol and triglycerides were reported in diabetic mice [63]. In this study administration of *S. caseolaris* moderately reduced these parameters (Table 10).

Table 8 Effect of *S. caseolaris* fruits on blood glucose level (mMol/L) in STZ-induced diabetic mice

Group	7th Days (mMol/L)	14th Days (mMol/L)	21th Days (mMol/L)	28th Days (mMol/L)
Control (2% tween solution, 0.5 mL/mice)	3.81 ± 0.20	4.15 ± 0.20	3.64 ± 0.10	3.92 ± 0.16
Diabetic control (2% tween, 0.5 mL/mice)	13.9 ± 0.36 [#]	13.5 ± 0.37 [#]	12.85 ± 0.40 [#]	12.65 ± 0.24 [#]
Standard (Glibenclamide, 10 mg/kg)	10.28 ± 0.27**	6.73 ± 0.18**	6.05 ± 0.23**	5.53 ± 0.32**
Test-I (EESC 300 mg/kg)	13.93 ± 0.43	12.81 ± 0.66	10.93 ± 0.47*	9.75 ± 0.30**
Test-II (EESC 500 mg/kg)	13 ± 0.41	11.72 ± 0.39*	8.93 ± 0.40**	7.6 ± 0.22**

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

[#]indicates $p < 0.0001$ compare with control

*indicates $p < 0.05$ compare with STZ control

**indicates $p < 0.0001$ compare with STZ control

Table 9 Effect of *S. caseolaris* fruits on body weight in STZ-induced diabetic mice

Group	7th day (gm)	14th day (gm)	21st day (gm)	28th day (gm)
Control (2 % tween solution, 0.5 mL/mice)	22.83 ± 0.40	24.33 ± 0.33	26.83 ± 0.40	30 ± 0.37
Diabetic control (2 % tween solution, 0.5 mL/mice)	23.33 ± 0.33	22.83 ± 0.60	21.5 ± 0.72 [#]	20.17 ± 0.6 [#]
Standard (Glibenclamide, 10 mg/kg)	23.67 ± 0.67	27 ± 0.63*	30.83 ± 0.6**	34.67 ± 0.67**
Test-1 (EESC 300 mg/kg)	22.5 ± 0.43	23.43 ± 0.60	26.33 ± 0.92*	29.67 ± 0.76**
Test-2 (EESC 500 mg/kg)	22.83 ± 0.60	24.83 ± 0.60*	28.5 ± 0.99**	30.67 ± 1.02**

Values are expressed as mean ± standard error of mean (n = 6)

EESC Ethanolic extract of *S. caseolaris* (L.) Engl

Indicates $p < 0.0001$ compare with control

*Indicates $p < 0.05$ compare with STZ control

** Indicates $p < 0.0001$ compare with STZ control

This action of *S. caseolaris* renders its lipid lowering activity in diabetic condition and hence prevents diabetes associated complications. The another findings of this study is that the extract not only shown the beneficial effect on blood glucose level but also reduced the urine glucose level in diabetic mice at the end of the experiment (Table 11). The results obtained from these studies are quite comparable with standard drugs glibenclamide and another similar studies on methanolic extract of this plant's fruit [64]. It is noteworthy that the authors in that article performed OGTT test only and here we have conducted both the OGTT and STZ induced diabetic model for confirming the antidiabetic properties of ethanolic extract of same plant part. Moreover the cited work was done up to 400 mg/kbw and herein we did it upto the dose level 500 mg/kbw. In cited study blood was sampled after 120 min (once) of glucose gavaging but herein we sampled for 3 times after 30, 90,150 min. It may be hypothesized that the result would be more reproducible as well as

reliable. The preliminary phytochemical screening indicated the presence of total phenolics, flavonoids, alkaloids and other secondary metabolites in considerable amounts in *S. caseolaris*. All of these compounds have been reported to possess strong antidiabetic potential [65, 66].

The individual phenolic compounds in the ethanolic extract of *S. caseolaris* fruit was standardized by HPLC. The HPLC separations of polyphenols in standard and EESC were shown in (Figs. 2 and 3) respectively. The content of each phenolic compound was calculated from the corresponding calibration curve and presented as the mean of five determinations. The experimental results indicated that the crude extract contained ellagic acid, vanillic acid and myricetin at 27.41, 3.06 and 7.93 mg/100 g of dry extract respectively. Since the phenolic compounds have been of interest against allergy and diabetes, the present HPLC study could be a potential mean to identify and quantify the polyphenolic compounds in the extracts targeting allergy and diabetics.

Table 10 Effect of *S. caseolaris* fruits on SGPT, SGOT, total Cholesterol, triglyceride, creatinine, urea and bilirubin in STZ-induced diabetic mice

Group	Control (2 % tween, 0.5 mL/ mice)	Diabetic Control (2 % tween, 0.5 mL/ mice)	Standard (Glibenclamide 10 mg/kg)	Test-I (EESC, 300 mg/kg)	Test-II (EESC, 500 mg/kg)
SGPT (U/L)	42.84 ± 5.71	81.84 ± 5.23 [#]	45.17 ± 4.94**	69.33 ± 3.75	55.50 ± 5.24*
SGOT (U/L)	45.84 ± 6.07	110.84 ± 6.32 [#]	51.67 ± 4.15**	93.67 ± 3.25*	56.33 ± 5.57**
Cholesterol (mg/dL)	119.0 ± 5.32	183.50 ± 8.28 [#]	111.50 ± 3.88**	177.33 ± 5.43	131.27 ± 7.89*
Triglyceride (mg/dL)	95.00 ± 6.73	172.83 ± 8.43 [#]	93.12 ± 4.71**	168.50 ± 4.02	125.83 ± 8.21*
Creatinine (mg/dL)	0.63 ± 0.027	3.25 ± 0.14 [#]	0.96 ± 0.07*	1.44 ± 0.08*	1.20 ± 0.05*
Urea (mg/dL)	29.00 ± 2.89	98.00 ± 2.67 [#]	34.00 ± 2.41*	47.67 ± 1.78*	36.67 ± 2.32*
Bilirubin (mg/dL)	0.43 ± 0.02	2.60 ± 0.06 [#]	0.61 ± 0.03*	1.12 ± 0.06*	0.93 ± 0.03*

Values are expressed as mean ± standard error of mean (n = 6)

Indicates $p < 0.0001$ compared with control

* Indicates $p < 0.05$ compare with STZ control

** Indicates $p < 0.0001$ compare with diabetic control

Table 11 Effect of *S. caseolaris* fruits extracts on urine glucose (mg/dL) in STZ-induced diabetic mice

Group	Normal control (2 % tween solution, 0.5 mL/mice)	Diabetic control (2 % tween solution, 0.5 mL/mice)	Standard (Glibenclamide 10 mg/kg)	Test-I (EESC 300 mg/kg)	Test-II (EESC 500 mg/kg)
7th days	-	+++	+++	+++	+++
14th days	-	+++	++	++	++
21st days	-	+++	+	+	-
28th days	-	+++	-	-	-

- = Not contain; + = 250 mg/dL; ++ = 500 mg/dL and+++ = 1000 mg/dL

Conclusions

The present study was carried out to evaluate the safety, anti-allergic, anti-hyperglycemic and profiling of bioactive polyphenols of *S. caseolaris* fruits extract. The HPLC analysis exhibited the presence of phenolic compounds like vanillic acid, myrecitin and ellagic acid. The fruit extract was found nontoxic in treated mice. The extract significantly improved the allergic symptoms as well as reduced the WBC count in blood. It also showed potentiality against diabetes. These findings may further be utilized as reference to isolate and characterize the pure compounds responsible for such activities.

Abbreviations

EESC: Ethanolic extract of *Sonneratia caseolaris* (L.) Engl.; TDI: Toluene 2, 4-diisocyanate; STZ: Streptozotocin; OECD: Organization for Economic Co-operation and Development; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; DMSO: Dimethyl sulfoxide; S.E.M.: Standard Error of Mean; p.o.: Per oral; vs: Versus

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Authors' contributions

The submitted research work was conducted in collaboration with the authors. The project was designed by AKD, SD and NNB. MAAB, KA and SA conducted the detail literature review and performed the phytochemical screening. MAAB, KA, SA and RNA have performed the extraction, acute and sub-acute toxicity tests, anti-allergic and anti-hyperglycemic activities. HPLC analysis was performed by HH. SD carried out the statistical analysis. Article was written by RNA, SD and NNB. Critical revision of the article was done by KKS, NNB and AKD. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article have been included within the article.

Declarations

Ethics approval and consent to participate

In our study, Organization for Economic Cooperation and Development guidelines for the care and use of animals were followed. Our study was approved by the Research Ethics Committee of Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh. Three membered ethics committee consists of Dr. Asish Kumar Das, Professor and

Chairman of the committee (dasasish03@yahoo.com), Dr. Jamil Ahmed Shilpi (jamilshilpi@yahoo.com), Professor and Dr. Sheikh Jamal Uddin, Associate Professor (uddinsj@yahoo.com); Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh. The approval number was KU/PHARM/AEC/15/06/026.

Consent for publication

All co-authors have consented for the publication of this manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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