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Original Research Article

Evaluation of Anti-Diabetic and Anti-Allergic Activities of *Brownlowia tersa* (L.) Kosterm Leaves Extract and Determination of its Phenolic Compounds by HPLC-DAD

Brototi Chakrabarty[1], Rabindra N. Acharyya[1], Neloy Kundu[1], Imran Mahmud[1,2], Hamayet Hossain[3], Nripendra N. Biswas[1], Shaikh J. Uddin[1], Shrabanti Dev[1]*, Asish K. Das[1]

[1]*Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh*
[2]*Department of Pharmacy, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh*
[3]*BCSIR Laboratories, Dhaka-1205, Bangladesh*

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ABSTRACT

Brownlowia tersa (L.), family Malvaceae, a shrub from Sundarbans (the largest Mangrove Forest of the World) has been traditionally used by the surrounding peoples for different ailments like inflammation, pain, diarrhea, allergy, etc. In this study, we investigated the anti-diabetic and anti-allergic activities of ethanol leaves extract of this plant. Oral glucose tolerance test (OGTT) was used to screen the anti-hyperglycemic activity. Streptozotocin (STZ)-induced diabetic mice were used to investigate the anti-diabetic activity. Anti-allergic activity was checked by using Toluene 2,4-diisocyanate (TDI)-induced allergic mice model by assessing allergic symptoms and WBC counts. HPLC-DAD analysis was used to detect the selected phenolic compounds. In OGTT, the extract showed a significant anti-hyperglycemic activity at 300 and 500 mg/kg doses in glucose loaded mice. In STZ-induced diabetic mice, the extract at both doses significantly reduced the blood and urine glucose levels. In addition, biochemical parameters such as serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), creatinine, bilirubin, urea, and triglycerides levels were also decreased. The extract at 300 and 500 mg/kg doses also significantly reduced the TDI induced allergic symptoms like sneezing ($P < 0.05$), scratching ($P < 0.05$) and nasal score ($P < 0.05$) and blood WBC counts. HPLC analysis revealed the presence of ten phenolic compounds which might be responsible for the reported activities in this study.

Keywords: *Brownlowia tersa*, Atreptozotocin, anti-diabetes, Anti-allergy.

Introduction

Brownlowia tersa (L.), a mangrove plant is locally known as Sundori lata.[1] The leaves of *Brownlowia tersa* (L.) possess anti-inflammatory, antioxidant, analgesic, anti-nociceptive and antidiarrheal activities.[2] The number of people suffering from diabetes mellitus has been increasing dramatically over the past few decades, and this demands special attention towards its management. WHO has suggested the evaluation of traditional plant treatments for diabetes as they are effective, nontoxic, with less or no side effects, and are considered to be excellent candidates for oral therapy.[3] Diabetes Mellitus (DM) is a metabolic disorder which is characterized by the presence of chronic hyperglycemia either immune-mediated (Type 1 diabetes), insulin resistance (Type 2), gestational or others (environment, genetic defects, infections, and certain drugs. Glucose utilization and endogenous glucose production or dietary glucose delivery are required for maintenance of a normal plasma glucose concentration.[4] The oral glucose tolerance test (OGTT) measures the body's ability to use glucose which is the body's main source of energy. Pre-diabetes and diabetes can be diagnosed by using an OGTT.[5] Streptozotocin (2-deoxy-2 (3-(methyl-3-nitrosoureido) -D-glucopyranose) is used to induce type 1 diabetes mellitus, T_1DM model (insulin-dependent diabetes mellitus, IDDM) which is associated with pronounced insulitis.[6]

Allergy is an immune-mediated inflammatory response to common environmental allergens including pollen, grasses, dust, and some medications.[7] Industrial chemical such as Toluene 2,4-diisocyanate (TDI), is one of the leading causes of occupation-related allergic diseases. TDI is considered to be a causative agent for allergic respiratory diseases observed among workers who are exposed to the vapour of this chemical in the industrial setting.[8] The major symptoms are reflected with various skin abnormalities such as allergic rhinitis, scratching, sneezing, rhinorrhea, swelling and redness.[9] The currently available antihistamine drugs are used for the treatment of allergic diseases, but these drugs have many adverse effects including somnolence, headache, body-weight gain, cardiac diseases, etc.[10]

Some plants are used in traditional medical practice for the treatment of allergy and diabetes since ancient times. The plants show anti-allergic and anti-hyperglycemic activity due to the presence of different secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and polyphenols.[11] HPLC-DAD analysis provided excellent identification and quantification of phenolic compounds.[12] The plants rich with polyphenols can act as anti-diabetic and anti-allergic activities.[13] The study investigated the anti-diabetic and anti-allergic activities of *Brownlowia tersa* (L.) leaves extract.

*Corresponding author. E mail: dshraban@yahoo.com
Tel: +88-01920514234

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Materials and Methods

Chemicals and Reagents

Streptozotocin (STZ) was purchased from Sigma-Aldrich, Germany and Toluene 2,4-diisocyanate (TDI) from Wako Chemical, Tokyo, Japan. The standard drugs; cetirizine and glibenclamide were obtained from Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh. HPLC grade acetonitrile, methanol, acetic acid and ethanol were obtained from Merck (Darmstadt, Germany).

Collection and preparation of ethanol extract

The leaves of *Brownlowia tersa* were collected from Munsiganj, Satkhira, Bangladesh during December, 2017 and were identified in the Bangladesh National Herbarium (BNH) Mirpur, Dhaka (Voucher specimen number-DACB-43583). The dried leaves were ground into powder of which 450 g was macerated in 2.25 L ethanol (95%). The maceration was for a period of 14 days with occasional shaking and stirring. It was then filtered, dried the filtrate, and an adhesive mass was obtained (Yield = 5.7%). The ethanol extract of *Brownlowia tersa* (L.) leaves was designated as EEBT.

Phytochemical screening

Qualitative phytochemical screening of the crude extract was carried out using standard method[14] for the identification of different chemical constituents.

HPLC detection and standardization of crude extract

Preparation of working standard solutions

Selected phenolic compounds in the crude extract were detected and quantified by HPLC-DAD analysis as previously described.[15] Briefly, 16 phenolic compounds were dissolved in methanol in a 25 mL volumetric flask to produce standard solutions. The concentrations of these solutions ranged from 4.0 to 50 µg/mL.

HPLC analysis

HPLC analysis was performed on a Shimadzu (LC-20A, Japan) equipped with a binary solvent delivery pump (SIL-20A HT), an auto sampler (SIL-20A HT), column oven (CTO-20A) and a photodiode array detector (SPD-M20A) and controlled by the LC solution software. The separation was performed using Luna C_{18} (5 µm) Phenomenex column (4.6 x 250 mm) at 33ºC. The mobile phase designated as A (1% acetic acid in acetonitrile) and B (1% acetic acid in water) with gradient elution.

*Experimental **animal***

Young Swiss albino mice aged 5-6 weeks (20-25 g) were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. They were kept in standard environmental condition for one week in the animal house of Pharmacy Discipline, Khulna University, Bangladesh for acclimatization. The standard guidelines of Animal Ethics Committee, Khulna University, were followed strictly for caring and handling of experimental mice (approval reference number was KUAEC-2018/07/12).

Acute toxicity study

Acute toxicity study was carried out following the procedure as previously described.[16] 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. The control group was treated with 2% tween 80 in water while test-I, test-II, test-III and test-IV groups received extract at doses of 500 mg/kg, 1000 mg/kg, 2000 mg/kg and 3000 mg/kg body weight. Animals were observed individually during the first 30 min after administration and then at every 24 h for 14 days for any clinical sign of toxicity (behavioural) or mortality.

Anti-diabetic activity test

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was carried out as per the procedure previously described with minor modifications.[17] The mice (12 hours fasted) were allotted randomly and divided into four groups of six mice each. Group-I was administered 2% tween water solution; group-II, glibenclamide 10 mg/kg and group-III, group -IV EEBT at 300 and 500 mg/kg, respectively. Dose of EEBT was selected as per previous study.[1,2] After 30 minutes, all groups received 10% glucose solution (2 g/kg body wt.). The blood glucose level was determined in by using the glucometer (Accu-check Active, Model No.: 0665619021) and expressed in millimole per liter (mmol/L).The blood sample was collected by piercing the tail tips with lancet and measured at 0 minutes, 30 minutes, 90 minutes and 150 minutes after glucose administration.

Streptozotocin (STZ)-induced diabetic model

Experimental design

STZ was given to the mice according to established methods with slight modification.[18] STZ (120 mg/kg) was dissolved in isotonic solution (0.9% NaCl) and injected intraperitoneally. After 72 hours, the blood glucose level was measured and mice having glycosuria and hyperglycemia (>10 mMol/L) were considered as diabetic mice and were used for the experiment. Mice were divided into five groups, consisting of six mice in each group. Group I (control, normal mice); group II (diabetic control, diabetic mice) were administered 0.5 mL 2% tween in water solution; group III (standard, diabetic mice) were administered 10 mg/kg glibenclamide solution and groups IV and V (test groups, diabetic mice) were administered 300 and 500 mg/kg EEBT, respectively for 28 days. Blood glucose, body weights and urine glucose level were measured on 7th, 14th, 21st and 28th day.[19] At the end of the experiment, the mice were sacrificed[20] and blood was collected for assessing the SGOT, SGPT, serum urea, serum creatinine, serum bilirubin, serum cholesterol, and triglycerides levels.[21]

Anti-allergic activity test

Experimental procedure

Anti-allergic test was carried out as per the procedure previously described with slight modifications.[22, 23] Experimental mice were randomly selected and divided into five groups, consisting of six mice in each group. Group-I (negative control) and Group-II (TDI control) were administered 0.5 mL solution of 2% tween-80 in water from day 1 to the 21st day. Group-III (standard group) was given antihistamine (cetirizine 20 mg/kg b.wt.) orally on the 21st day. Group-IV and Group-V were administered EEBT (300 and 500 mg/kg body weight respectively) everyday. Dose of EEBT was selected as per previous study.[1, 2] TDI sensitization was done one hour after the oral administration of drug and EEBT. Group I was sensitized with only ethyl acetate (10 µL) bilaterally at both nasal vestibules but all other four groups were sensitized with 10 µL of 5% TDI solution in ethyl acetate for five consecutive days. After the 2-day interval, it was repeated for five consecutive days. After nine days of 2nd sensitization each mouse was provoked in the same manner (Figure 1).

Assessment of allergy-like symptoms

Nasal allergy–like symptoms such as the number of sneezes, scratch and nasal score were measured immediately just after TDI treatment for 10 minutes by placing the animals in separate cages.[23, 24]

Blood sample collection and differential analysis

Mice were anesthetized and blood was collected from the cervical vein as previously described.[25] The blood was collected into a heparinized tube and used for total and differential leukocyte count. Cells were identified as eosinophils, lymphocytes, monocytes, neutrophils or basophils.

Statistical analysis

Results are presented as mean ± SEM. Statistical analysis of all the data obtained was evaluated by using Student's *t*-test Calculator and Graphical presentation was carried out in Microsoft Excel 2013. The results were considered statistically significant when $P < 0.05$.

Results and Discussion

Phytochemical screening

Plant antioxidant and metabolite have been reported to possess anti-diabetic and anti-allergic potentials.[28] Phytochemical screening of the crude ethanol extract of *B. tersa* leaves demonstrated the presence of different phytochemical groups such as combined reducing sugar, tannins, flavonoids, saponins, steroids, alkaloids, glycosides and terpenoids, and absence of gums, proteins and acidic compounds. Presence of these active metabolites in considerable amounts justify the possibility of anti-allergic and anti-diabetic potentials of *B. tersa.*

HPLC analysis

Flavonoids originated from foods could improve glucose metabolism. Plants are rich sources of polyphenols, HPLC-DAD analysis provided identification and quantification of ten phenolic compounds present in the ethanol extract of *B. tersa* leaves which include a moderate concentration of catechin hydrate, catechol, (-) epicatechin, caffeic acid, *p*-coumaric acid, trans-ferulic acid, myricetin, quercetin, trans-cinnamic acid and kaempferol (Figures 2 and 3, Table 1). The flavonoid-*O*-diglycoside group contained compounds, such as quercetin, kaempferol, and myricetin also known to have anti-diabetic and anti-allergic activities.[29,30] Tannins have been observed to enhance the glucose uptake through mediators of the insulin-signaling pathways, such as $P^{I3}K$ (Phosphoinositide 3-Kinase) and p^{38} MAPK (Mitogen-Activated Protein Kinase) activation and GLUT-4 translocation.[31] Alkaloids exert a wide range of anti-diabetic and anti-allergic activities through different mechanisms.[32,33]

Acute toxicity study

In the acute toxicity test, there was no lethality or any toxic reactions found within the experimental period at any of the doses up to 3000 mg/kg, suggesting the LD_{50} of the extract is above 3000 mg/kg. This result indicates the safely of this plant for therapeutic use.

Anti-diabetic study

Oral glucose tolerance test

Oral glucose-induced hyperglycemia and the investigation of the ability of any agent to reduce this increased glucose level may be used to investigate new anti-hyperglycemic drug. Oral hypoglycemic agents should lower the blood glucose levels effectively.[34] In OGTT, the leaves extract showed reductions in blood glucose level compared to control group mice (Table 2). This result led us to go ahead with further experiment to confirm its anti-diabetic potential.

STZ induced diabetic mice

STZ-induced diabetes is characterized by increasing glucose level and a severe loss in body weight, polyphagia and polydipsia.[35] Streptozotocin damages the pancreatic *β*-cell which is responsible for insulin secretion and causes experimental type 1 diabetics. The administration of Streptozotocin markedly increased the blood glucose level of the diabetic control, standard and tested group mice compared to the control group. Ethanol extract of *B. tersa* decreased the elevated blood glucose level in the blood sample of mice (Table 3) at both doses. Again urine analysis on 7^{th} day showed the presence of glucose in the entire group, except control group. But the continuous administration of the ethanol extract of *B. tersa* reduced the urine glucose level in diabetic mice when compared to diabetic control mice (Table 4). On the other hand, normal mice gained weight whereas diabetic mice lost their weight gradually due to catabolic reaction. Treatment with glibenclamide and EEBT at both doses protected the gradual weight loss rather than increase the gradual weight gain (Table 5). Biochemical analysis also revealed that the diabetic control mice showed a significant increase in different biochemical parameters like SGPT, SGOT, triglycerides, cholesterol, creatinine, urea and bilirubin compared with control. But oral administration of ethanol extract of *B. tersa* resulted in a significant ($P < 0.05$) reduction in the above mentioned parameters (Table 6).

Muscle wasting which occurred by catabolic reactions might be the cause of weight reduction in diabetic mice. Oral administration of the extract moderately improved the body weight in diabetic mice. Furthermore, elevated enzymes levels (SGPT, SGOT, bilirubin, cholesterol, triglycerides, etc.) were associated with the increased risk of diabetes.[36] The extract treated mice showed significant decrease of these biochemical parameters. Various studies showed that quercetin[37], myricetin[38] and derivative of catechol, catechin[39] have significant anti-diabetic activity. The HPLC analysis, ensured the presence of these compounds in EEBT.

Anti-allergic activity

In anti-allergic activity evaluation, EEBT significantly ($P < 0.05$) suppressed TDI-induced allergy-like symptoms like the number of sneezes, scratches and the nasal score in a dose-dependent manner. In TDI-sensitized mice, the total number of sneezes, scratches and the nasal score were 32.5 ± 4.4, 286.33 ± 23.21 and 2.95 ± 0.28, respectively. Whereas the number of sneezes, scratches and nasal score, for standard cetirizine were 11.5 ± 1.78, 114.33 ± 12.73 and 0.533 ± 0.15 (Table 7). Oral administration of EEBT significantly decreased the number of sneezes, scratches and the nasal score (Table 7).

In the blood samples of TDI-control group mice, the total numbers of circulating leukocytes, eosinophils, lymphocytes and basophils were markedly increased when compared to the control mice, although the numbers of monocytes and neutrophils were not significantly different. In contrast, oral administration of extract significantly decreased the total and differential counts of WBC in a dose-dependent manner when compared to the TDI control mice (Table 8).

The current therapies for allergic diseases focus primarily on control of symptoms and suppression of inflammation.[40] Anti-allergic agent's act by preventing the release of inflammatory mediators or inhibiting the actions of released mediators on their target cells. It has been reported that sneezing and nasal rubbing are caused by histamine through its binding to histamine H_1 receptor (H_1R) on the sensory nerve endings.[42] H_1R plays a key role in histamine signaling involved in the allergic response. Flavonoids inhibit histamine release, synthesis of IL-4 and IL-13 and CD_{40} ligand expression by basophils.[43] Like flavonoids, steroids also have the ability to reduce allergic symptoms.[44] Additionally, the study showed that the total leukocytes, lymphocytes, neutrophils eosinophils and monocytes increased in the blood of TDI-control mice. This eosinophilia and increased leukocytes act as a good cellular biomarker for allergic sign.[45] Oral administration of EEBT at 300 and 500 mg/kg significantly reduced the total count of WBC cells as compared to TDI-control. Myricetin, quercetin, catechin and (-) epicatechin were promising anti-allergic agents found in various reports[45, 46] which are present in EEBT. Compounds identified through HPLC analysis might be responsible for the anti-diabetic and anti-allergic activity of this extract.

Table 1: Polyphenolic compounds in the ethanol extract of *B. tersa* leaves

Phenolic Compounds	Contain in (mg/100 g dry *B. tersa* extract)
Catechin hydrate	76.99
Catechol	101.35
(-) Epicatechin	35.19
Caffeic acid	17.16
p-Coumaric acid	156.99
trans-Ferulic acid	55.19
Myricetin	19.83
Quercetin	74.65
trans-Cinnamic acid	4.45
Kaempferol	36.28

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Table 2: Hypoglycemic effect of *B. tersa* leaves on oral glucose loaded mice

Group	Blood glucose level (mMol/L) + SEM			
	Fasting stage	30 min	90 min	150 min
Control, 2% tween in water	4.38 ± 0.48	14.04 ± 0.12	8.42 ± 0.34	6.06 ± 0.31
Glibenclamide 10 mg/kg)	3.8 ± 0.29	4.72 ± 0.24*	3.76 ± 0.40*	3.12 ± 0.29*
EEBT 300 mg/kg	3.7 ± 0.23	8.1 ± 0.39*	7.64 ± 0.31†	5.94 ± 0.40†
EEBT 500 mg/kg	4.78 ± 0.26	8.7 ± 0.35*	6.82 ± 0.29†	4.82 ± 0.37†

Values are expressed as mean ± standard error of mean (n = 6). *P < 0.001 when compared with control; $^{\dagger}P$ < 0.05 compared with control.

Table 3: Effect of *B. tersa* leaves on blood glucose level in STZ-induced diabetic mice

Group	Blood glucose level (mMol/L) + SEM			
	7th day	14th day	21st day	28th day
Control (0.5 mL, 2% tween water solution)	3.81 ± 0.20	4.15 ± 0.20	3.64 ± 0.10	3.92 ± 0.16
Diabetic control (0.5 mL, 2% tween water solution)	13.9 ± 0.36*	13.5 ± 0.37*	12.85 ± 0.40*	12.65 ± 0.24*
Glibenclamide 10 mg/kg	10.28 ± 0.27‡	6.73 ± 0.18‡	6.05 ± 0.23‡	5.53 ± 0.32‡
EEBT (300 mg/kg)	13.6 ± 0.29	12.43 ± 0.22†	10.87 ± 0.52†	9.17 ± 0.25‡
EEBT (500 mg/kg)	12.15 ± 0.27†	11.1 ± 0.29†	9.13 ± 0.28‡	7.65 ± 0.30‡

Values are expressed as mean ± standard error of mean (n = 6). *P < 0.001 when compare with control; $^{\dagger}P$ < 0.05 when compared with diabetic control; $^{\ddagger}P$ < 0.001 compared with diabetic control.

Table 4: Effect of *B. tersa* leaves on urine glucose in STZ-induced diabetic mice

Group	Urine glucose level (mg/dL)			
	7th day	14th day	21st day	28th day
Control (2% tween water solution)	-	-	-	_
Diabetic control (2% tween water solution)	+++	+++	+++	+++
Glibenclamide (10 mg/kg)	+	+	_	_
EEBT (300 mg/kg)	++	++	+	+
EEBT (500 mg/kg)	++	+	+	+

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

Table 5: Effect of *B. tersa* leaves on body weight in STZ-induced diabetic mice

Group	Body weight (g) ± SEM			
	7th day	14th day	21st day	28th day
Control (2% tween water solution)	22.83 ± 0.4	24.33 ± 0.33	26.83 ± 0.4	30 ± 0.37
Diabetic control (2% tween water solution)	23.33 ± 0.33	22.5 ± 0.43	21.5 ± 0.72*	20.17 ± 0.6*
Glibenclamide (10 mg/kg)	23.67 ± 0.67	26 ± 0.37†	27.83 ± 0.31‡	30 ± 0.45‡
EEBT (300 mg/kg)	23.17 ± 0.31	25.17 ± 0.31†	27 ± 0.37‡	29 ± 0.37‡
EEBT (500 mg/kg)	23 ± 0.37	25.83 ± 0.31‡	29 ± 0.37‡	31.67 ± 0.42‡

Values are expressed as mean ± standard error of mean (n = 6). *P < 0.001 compare with control; $^{\dagger}P$ < 0.05 when compared with diabetic control; $^{\ddagger}P$ < 0.001 compared with diabetic control.

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Table 6: Effect of *B. tersa* leaves on SGPT, SGOT, Cholesterol, Triglyceride, S. Urea S. Creatinine and S. Bilirubin in STZ- induced diabetic mice

Biochemical parameters	Control (2% tween water solution)	Diabetic control (2% tween water solution)	Glibenclamide (10 mg/kg)	EEBT (300 mg/kg)	EEBT (500 mg/kg)
SGPT (U/L)	42.8 ± 5.71	81.84 ± 5.23*	45 ± 4.94‡	73.5 ± 5.22	53.83 ± 5.88†
SGOT (U/L)	45.84 ± 6.07	110.8 ± 6.32*	51.67 ± 4.15‡	94.4 ± 3.29†	57.5 ± 5.89‡
Cholesterol (mg/dL)	119 ± 5.32	183.5 ± 8.28*	111.5 ± 3.88‡	179.2 ± 5.881	129.8 ± 5.5‡
Triglyceride (mg/dL)	95 ± 6.73	172.83 ± 8.43*	93.12 ± 4.71‡	174 ± 4.41	124.4 ± 6.82‡
S. Urea (mg/dL)	29 ± 2.89	98 ± 2.67*	34 ± 2.41†	49.83 ± 3.0†	32.67 ± 3.18†
S. Creatinine (mg/dL)	0.63 ± 0.03	3.25 ± 0.143*	0.96 ± 0.07†	2.17 ± 0.27†	1.82 ± 0.5†
S. Bilirubin (mg/dL)	0.43 ± 0.02	2.6 ± 0.06*	0.61 ± 0.04†	2.35 ± 0.4†	1.68 ± 0.32†

Values are expressed as mean ± standard error of mean (n = 6). *P < 0.001 compared with control; †P < 0.05 compared with diabetic control; ‡P < 0.001 compared with diabetic control.

Table 7: Assessment of allergy-like symptoms in mice

Group	No. of sneezes	No. of scratches	Nasal score
Control (2% tween water solution)	0.50 ± 0.34	12 ± 2.1	0.00 ± 0.00
TDI control (2% tween water solution)	32.5 ± 4.40*	286.33 ± 23.21*	2.95 ± 0.28*
Cetirizine (20 mg/kg)	11.5 ± 1.78†	114.33 ± 12.73‡	0.53 ± 0.15†
EEBT (300 mg/kg)	22.17 ± 1.05†	193 ± 10.19†	2.17 ± 0.31†
EEBT (500 mg/kg)	15.5 ± 4.26†	134 ± 9.17‡	1.03 ± 0.212†

Values are expressed as mean ± standard error of mean (n = 6). *P < 0.001 compared with control; †P < 0.05 compared with TDI control; ‡P < 0.001 compared with TDI control.

Table 8: Total and differential analysis of blood

Group	× 10^3 cells /mL					
	TC (WBC)	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Control	8.33 ± 0.15	5.36 ± 0.17	2.47 ± 0.12	0.37 ± 0.07	0.02 ± 0.01	0.12 ± 0.05
TDI control	11.18 ± 0.12*	7.14 ± 0.11*	3.72 ± 0.10*	1.025 ± 0.02*	0.18 ± 0.04*	0.02 ± 0.01
Cetirizine (20 mg/kg)	8.97 ± 0.25‡	4.72 ± 0.15‡	2.81 ± 0.06‡	0.41 ± 0.05‡	0.04 ± 0.02†	0.09 ± 0.02†
EEBT (300 mg/kg)	8.7 ± 0.12‡	5.24 ± 0.13‡	2.74 ± 0.05	0.65 ± 0.02‡	0.47 ± 0.19	0.00 ± 0.00
EEBT (500 mg/kg)	5.3 ± 0.17‡	2.72 ± 0.08‡	2.39 ± 0.06‡	0.13 ± 0.01‡	0.13 ± 0.08	0.00 ± 0.00

Values are expressed as mean ± standard error of mean (n = 6).*P<0.001 compared with control; †P<0.05 compared with TDI control; ‡P < 0.001 compared with TDI control.

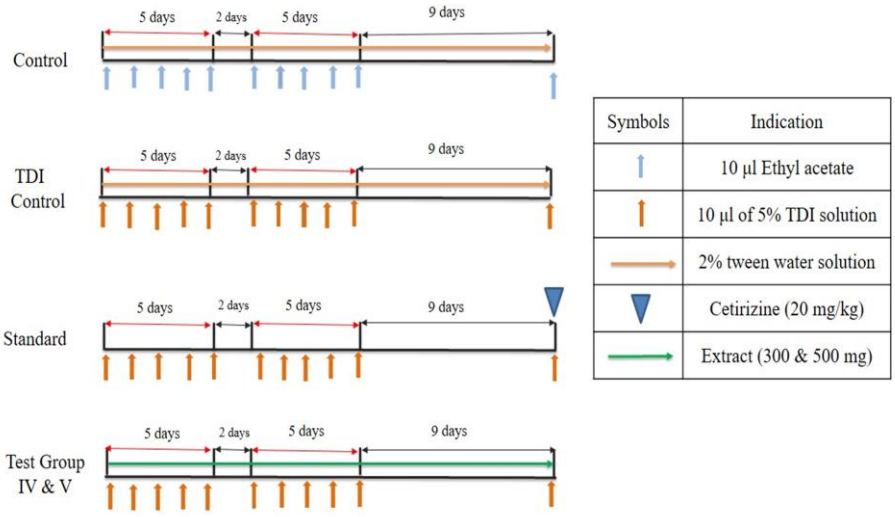

Figure 1: Experimental protocol for anti-allergic activity evaluation

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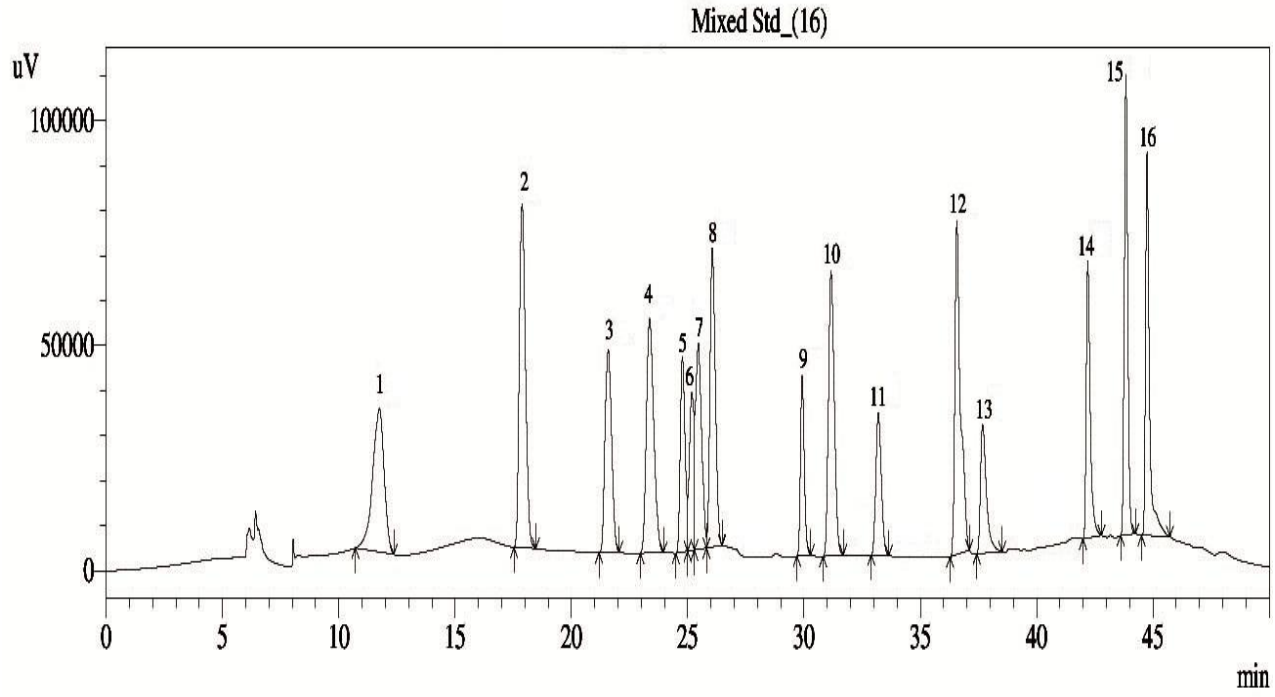

Figure 2: HPLC chromatogram of a standard mixture of polyphenolic compounds. Peaks: **1.** Gallic acid; **2.** 3,4 dihyrdoxy benzoic acid; **3.** Catechin hydrate; **4.** Catechol; **5.** (-) Epicatechin; **6.** Caffeic acid; **7.** Vanillic acid; **8.** Syringic acid; **9.** Rutin hydrate; **10.** *p*-Coumaric acid; **11.** Trans-Ferulic acid; **12.** Rosmarinic acid; **13.** Myricetin; **14.** Quercetin; **15.** Trans-Cinnamic acid, and **16.** Kaempferol**.**

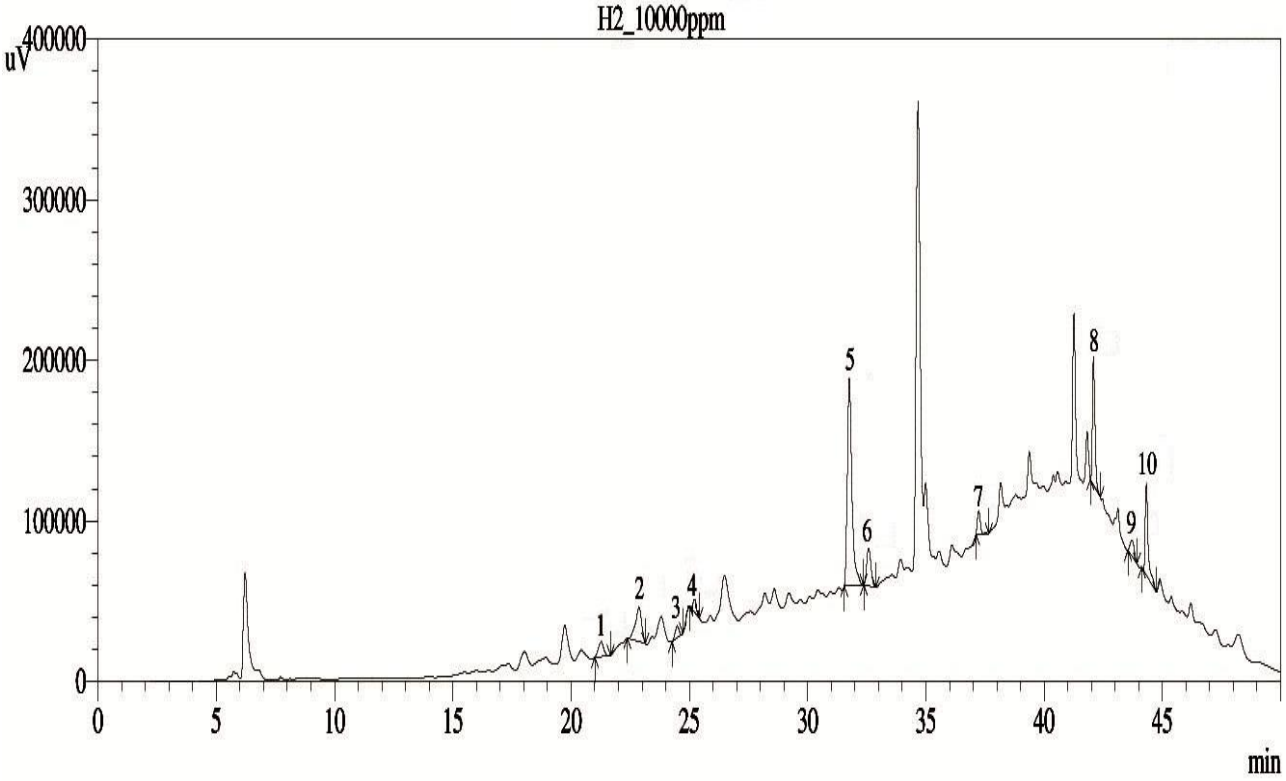

Figure 3: HPLC chromatogram of ethanolic extract of *B. tersa* leaves. Peaks: **1.** Catechin hydrate; **2.** Catechol; **3.** (-) Epicatechin; **4.** Caffeic acid; **5.** *p*-Coumaric acid; **6.** Trans-Ferulic acid; **7.** Myricetin; **8.** Quercetin; **9.** Trans-Cinnamic acid and **10.** Kaempferol.

Conclusion

The present study provides the scientific evidence for the anti-diabetic and anti-allergic activities of *Brownlowia tersa* (L.) leaves. The phytoconstituents like myricetin, quercetin, catechin and (-) epicatechin might be responsible for these activities. Further investigation will help to isolate and characterize the compounds targeting these diseases.

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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