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Antidiabetic and antiulcer activity of methanolic extract of *Tradescantia spathacea* in rats

Syeda Shaheen Sultana[1*], S Tasqeeruddin[2], Yahya I Asiri[3], NS Murali Krishna[1] and Syeda Nishath Sultana[4]

[1]School of Pharmacy and Medical Science, Singhania University, Pacheribari, Jhunjhunu, Rajasthan, India
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Abha, Saudi Arabia
[3]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia
[4]Department of Pharmacology, Sultan-Ul-Uloom College of Pharmacy, Banjara hills, Hyderabad, Telangana, India

Abstract: The present study was designed to assess *Tradescantia spathacea's* antidiabetic ability, as well as the antiulcer activity of the entire plant extract. The diabetic condition was evaluated using Streptozotocin's oral glucose tolerance test, diabetes-alloxan, and diabetes-models. Antiulcer activities were observed in rats where gastric ulcers were either caused by oral administration of ethanol, or pyloric ligation. Standards include ranitidine, glibenclamide and sucralfate. In all models, the blood glucose levels of animals treated with the test extract were found to be significantly lower compared to diabetic care. Similarly, in all models, the ulcer index in the animals treated with the test extract was found to be significantly lower relative to the animals under vehicle supervision. Our findings say *T. Spathacea* extract has essential anti-diabetic properties, as well as antiulcer properties.

Keywords: *Tradescantia spathacea*., alloxan induced diabetes, streptozotocin-induced diabetes models, ranitidine, sucralfate, glibenclamide

INTRODUCTION

Diabetes mellitus is an extreme progressive systemic condition that is a significant cause of illness worldwide. Hyperglycemia disorder is characterized by metabolic conditions leading to absolute or relative depletion of insulin hormones. Hyperglycemia caused many other factors, including dyslipidemia or hyperlipidemia, including the prevalence of micro and macro vascular diabetes disorders, which are the major causes of morbidity and mortality, in addition to hyperglycemia (Owusu *et al*., 2020). According to World Health Organization (2010) estimates, the prevalence of diabetes is expected to rise by 35 percent by the end of 2020. There are over 150 million diabetics worldwide currently, and by 2025, this is expected to increase to 422 million or more. The Indian statistical prediction reveals that the number of diabetics (www.who.int/health-topics/diabetes) will grow from 15 million in 1995 to 57 million in 2025, the highest number of diabetics in the world. Reasons for this rise include improved living conditions, a sedentary lifestyle, energy-rich food consumption, obesity and a longer life span among others. Many countries in Asia and Africa have the highest number of diabetics, where diabetes mellitus prevalence could rise to 2-3 folds relative to current levels. It is increasingly important to test plant products for the treatment of diabetes mellitus, as they contain multiple therapeutically feasible bioactive compounds. While a large number of medicinal plants have already been studied for their antidiabetic effectiveness, there is still a need to research many other medicinal plants.

Peptic ulcer disease typically refers to a category of complications in any component of g.i. marked by ulcer presentation. Exposure to acid for a sufficient period and dosage, this ulceration occurs most commonly in the small intestine (duodenal ulcer) or stomach (gastric ulcer) (Scarpignato *et al*., 2016). The mucosal damage is caused by H. *pylori* or NSAID. In animals with gastric acid, the production of gastric acid tends to be normal or decreasing. There may be degradation of the mucosal defense factor when gastric acid expands in the presence of reduced acid (White *et al*., 2015; Scheiman 2016).

Tradescantiaspathacea Swartz [synonyms: Discolor rhoeo L. H'erHance, Rhoeospathacea (Swartz) Stearn], the Commelinaceae family (Argueta Villamar *et al.,*1994; Tirumala *et al.,* 2018). The decoction of *T. Spathacea* leaves is openly consumed as a curative of cancer daily, without existing scientific proof of such property in both India and South East Mexica. *T. Spathacea* the aqueous extract used in the treatment *of* blocks bretylium's antiadrenergic action (Tirumala *et al.,* 2018). Extracts from *T. Spathacea* were used in cosmetics to enhance skin appearance (Idaka *et al.,* 1987). Some experimental documents reported that flavonoids, anthocyanins, saponins, carotenoids, waxes, terpenoids, and derivatives of coumarin and steroids found in *T. Spathacea*are (Gonzalez avila *et al.,* 2003). Conversely, *T. Spathacea* crude methanolic extract, tested in an *in vitro* setting, showed antioxidant activity (Gonzalez-avila *et al.,* 2003)

**Corresponding author:* e-mail: sultanashaheen836@gmail.com

and antimicrobial properties (Parivuguna., 2008). The importance of this species exploration is noticeable, but the lack of scientific reports *in vivo* which corroborate *T. Spathacea* property of Antidiabetic and Antiulcer Activity,

MATERIALS AND METHODS

Animals

Animal experiments were performed on albino rats weighing about 200-250g. Divided into 5 groups of 6 animals per cage. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24 hrs). The food in the form of dry pellets and water was provided using *ad libitum*. All the animals were approved by the institutional ethics approval committee.

Chemicals

Alloxan-induced Diabetic model used chemical was Alloxan monohydrate marketed sample of Qualikems fine chemical Pvt. Ltd, Streptozotocin marketed sample of Cayman Chemicals (USA), Glibenclamide marketed sample of Sanofi India Ltd (Mumbai), Dextrose marketed sample of Triveni chemicals, Jeecon food Pvt Ltd (Delhi) Glucometer, Blood Gluco-strips one-touch-horizon, marketed by Johnson & Johnson Ltd. All other chemicals and reagents were used with the analytical standard.

Plant material

The plant *Tradescantia spathacea* was collected from sultan-ul-Uloom College of pharmacy medicinal gardens in Hyderabad, Telangana state, India within November 2019.Prof. Rana Kausar, Dept, had described the plant specimen of Botany, University of Osmania, Hyderabad, State of Telangana, India (voucher specimen number 0364).

Preparation of hydroalcoholic extract

T. spathacea plant was harvested at 35-40°C each week, washed thoroughly in water, chopped, and air-dried. This was then pulverized into an electric grinder to produce a fine powder. The obtained powder was extracted with petroleum ether and subsequently, using a 70 percent methanol singing soxhlet apparatus. Extract (METS) was washed under reduced pressure, condensed, and dried.

Experimental models

Check for oral glucose tolerance

All of the fasting rats were split into 5 groups of 6 animals per cage. The community I served as a standard monitor and the rats in both grades were orally administered with 2g/kg of glucose after 30 minutes of extract administration. Just before glucose was administered and at 30, 60, 90 and 120 min after glucose processing, blood samples from the rat tail vein were collected. A glucometer was used to immediately measure blood glucose levels (Jagannathan *et al*., 2020).

Diabetic model of alloxan-induced

Alloxan monohydrate was first individually weighted by the weight of each animal and then, before being administered, solubilized with 0.2ml of saline only. By administering it at a dosage of 150mg/kg b Wt. Intraperitoneal. The animals were given feed ad libitum after 1 hour of administration of alloxan and 5 percent dextrose solution was also given for one day in the feeding bottle to overcome the early hypoglycemic phase. The animals were placed under control, and after 48 hours, blood glucose was measured by a glucometer. One party served as a monitor only for the vehicle. The diabetic rats (glucose level > 300 mg/dl) were isolated for laboratory testing and separated into six separate classes with six animals in each group. Class II was left untreated as diabetic monitors and functioned. Group III obtained 600µg/kg of glibenclamide, and Group IV rats were provided with T-methanol extract. A dosage of 200mg/kg b. wt, and Group V rats were treated with methanolic extract of *T. Spathacea* 400mg/kg b. wt. for 7 days (Manoharan *et al.,* 2009; Keita *et al*., 2020).

Diabetic model-induced streptozotocin

The rats were fasted 18 hours before diabetes induction, after 2 weeks of feeding with high-fat food, and injected with a single dose of streptozocin 60 mg/kg intraperitoneally, freshly dissolved in a normal saline solution. The rats had free access to food after administration (normal diet on pellets) and water ad libitum. In rats, extreme polydipsia and marked polyuria have created diabetes. After 3 days, i.e. 72hrs of injection, blood glucose was determined by a glucometer. Animals with blood glucose levels > 140 mg/dl are isolated and used for testing purposes. One group was functioning as a control unit for cars. Diabetic rats (glucose level > 300 mg/dl) were segregated and separated for laboratory testing into five different groups, each group having six animals. Class II remained untreated and functioned as a diabetes monitor. Glibenclamide, group IV of methanol extract treated rats. T Spathacea in Category III was 600 µg/kg. The dosage of spathacea was 200 mg/kg b. Wt; rats got methanol extract T from group V. About 400mg/kg b.Wt. The rats chosen were administered with the same research samples as before, but blood glucose levels were measured on treatment days 1, 3, 5, 7, 9 and 14. (Eidi A, 2006; Gheda *et al*., 2020)

Action for antiulcer

Form of pyloric ligation

In this process, albino rats have fasted for 36 hours in individual cages. Care was taken to discourage coprophagia. In each group, rats of either sex were randomly split into five groups of six males. Group-I: regular saline control, Group-II: ulcerative control (1% CMC, 5 ml/kg, p.o.), Group-III: ranitidine (30 mg/kg,i.p.), Group-IV: METS (200 mg/kg i.p.), Group-V: METS (400 mg/kg i.p.), Group-II: normal saline control, Group-II: ulcerative control (1% CMC, 5 ml/kg, p.o.). Test extracts

were administered 30 min before pyloric ligation, or reference medication or control vehicle. Under mild ether anesthesia, the belly was opened and the pylorus ligated. At the end of 19 h after ligation, gastric juice was obtained and volume determined. Then it was exposed to the glandular part and ulceration was studied (Agrawal R, 2010).

Methods of gastric cytoprotection (Ethanol-induced ulcers)

In normal conditions, 200-250g of albino rats are kept light and dark at 12 h. Food was available in the form of dry pellets and water. Rats of either sex of each group were randomly separated into five groups of six males. Group-I: daily saline therapy, Group-II: managed by ulceration (1 ml ethanol, p.o.), Group-III: controlled by sucralfate (400 mg/kg, p.o.), Group-IV: controlled by METS (200 mg/kg i.p.), Group-V: controlled by METS (400 mg/kg i.p.), Group-II: controlled by ulceration (1 ml ethanol, p.o.). Each rat received 1 ml of methanol orally for 30 minutes after the assessment or reference drug or the control vehicle treatment. After 1h with excess anesthetic ether, the rats were euthanized and the stomach was cut open along the greater curvature, the residual matter was removed with saline, and ulceration was examined on the inner surface (Al-Harbi *et al*., 1997)., The ulcer index, as well as the percentage protection against the ulcer, was calculated.

Determinations with different parameters

Ulcer index assessment (UI) (Jalilzadeh-Amin *et al*., 2015)

The ulcerative index was determined by the severity of the lesions of the gastric mucosa and rated as 1 mm or less of erosion, 1-2 mm and 1,2,3 or more than 2 mm respectively.

Gastric juice set

Gastric juice from ligated pylorus rats was extracted. For 10 minutes, the extracted gastric juice was centrifuged at 60 rpm and the amount of gastric juice was assessed (Khan *et al*., 2012).

Determination of acidity free and acidity complete

In a 100ml conical flask, 1ml gastric juice was taken, added to the 2-3 drops of topfer's reagent and titrated with 0.01 NaOH before all red color signs disappeared and the solution became the endpoint (yellowish orange). The volume of alkali added was noted. This volume corresponds to free acidity. Then 2-3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. The volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by using the formula:

Acidity (mEq/litre) = volume of NaOH
Normality of NaOH 100/0.1 liter = volume of NaOH
Normality of NaOH 100/0.1 liter of NaOH

STATISTICAL ANALYSIS

The results were statistically tested using the originPro8.5 tools in the one-way ANOVA test followed by Dunnet's test. The data were shown as mean ± SEM. At $P<0.05$, the minimum degree of significance was set.

RESULTS

Antidiabetic activity

Table 1-5 displayed data collected from normal, glucose-hyperglycaemic, and diabetic rats. Methanolic extracts containing *T. spathacea*, but there was no major activity found. As the comparison used, glibenclamide demonstrated substantial activity in the same state (table1). The groups treated with the extract showed significant results.

Test samples were administered over 7 days to alloxan-induced diabetic rats. Blood glucose levels and body weights were determined on the days 0hr, 1hr, 3hr, 5hr, 3day, 5day, and 7^{Th} day. The data indicate that *T. spathacea* is 400mg/kg.

During 14 days, test samples were given to streptozotocin-induced diabetic rats. The blood glucose concentrations and body weights were calculated on days 1, 3, 5, 7, 14. The data indicate that *T. spathacea* extract is 400 mg/kg, which has a remarkable effect on the amount of blood glucose equivalent to the reference drug (table 3).

Antiulcer activity

Pyloric ligation method

Ethanol-induced ulcers

Effect of T. spathacea extract in pylorus ligated rats

The ulcer was formed through pylorus ligation in all animals in the ulcerated control group, and the mean ulcer index was 10.25±0.891 suggesting the ulcerative effect. In comparison to normal regulation, another ulcerative effect was measured as follows (Tables 6 and 7); mean volume of gastric content as 6.18±0.17, pH as 1.67±0.05, free acidity as 0.252±0.009, gross acidity as 45.7±1.21, indicating production of ulcers in animals; pylorus ligation also produced ulcers in both. Compare to the pretreated animals with *T. spathacea* extract. However, the ulcer index reported a considerable dose-dependent decrease of 200 mg/kg (UI; 5.33±0.494) and 400 mg/kg (UI: 2.55±0.199) in animals pretreated with test extracts. It showed gastroprotection of 48.00 percent at 200 mg/kg as compared to ulcerated strength, and gastroprotection of 75.12 percent at 400 mg/kg.

Effect of T. spathacea extract in ethanol-induced gastric ulcer

The ethanol-induced ulcer control group developed ulcers in all animals and the mean ulcer index was 18.5±4.86, suggesting the ulcerative effect. However, a substantial dose-dependent decrease of 200 mg/kg (UI; 5.10±1.21)

Table 1: Effect of *T. spathacea* extract on oral glucose tolerance in rats

Group	Treatment	The blood glucose level in mg/dl				
		0 min	30 min	60 min	90 min	120 min
Group I	Normal control(Saline)	101.58 ± 4.53	163.84 ± 7.33	129.03 ± 5.28	110.13 ± 3.71	104.62 ± 3.81
Group II	Diabetic Control	367.97 ± 35.72	562.68 ± 27.47	528.72 ± 15.37	483.78 ± 16.76	454.71 ± 21.11
Group III	Glibenclamide	300.67 ± 27.42	479.22 ± 27.36	440.72 ± 16.54	422.78 ± 12.86	372.89 ± 19.70***
Group IV	METS (200mg/kg)	342.77 ± 16.44	507.92 ± 33.28	461.28 ± 28.10	421.40 ± 24.88	382.77 ± 29.53*
Group V	METS (400mg/kg)	308.77 ± 18.50	446.93 ± 22.20	429.66 ± 24.99	396.68 ± 14.76	379.52 ± 14.76**

Anti Diabetic effect of *T. spathacea* extract Values are mean ± SD, n = 6 in each group, *P<0.05, **P<0.01 when compared with the diabetic control group (oral glucose tolerance)

Table 2: Effect of *T. spathacea* extract on blood glucose levels (mg /dl) of alloxan-induced diabetic rats

Group	Treatment	Blood glucose level mg/dl						
		0 hr	1hr	3 hr	5 hr	3rd day	5th day	7th day
Group I	Normal Control (Saline)	80.00 ± 1.6	80.83 ± 1.72	80.83 ± 1.42	79.83 ± 1.37	81.33 ± 0.98	79.83 ± 0.83	81.16 ± 0.792
Group II	Diabetic Control	322.33 ± 7.77	327.50 ± 7.94	329.50 ± 7.38	336.67 ± 6.51	369.00 ± 6.11	388.33 ± 16.59	413.50 ± 4.75
Group III	Glibenclamide	277.33 ± 7.92	206.66± 6.28	174 ± 7.09	154.83 ± 5.04	125.33 ± 6.96	114.33 ± 5.25	105.66 ± 5.09**
Group IV	METS (200mg/kg)	348.5 ±11.73	311 ±10.51	295 ± 12.75	287.3 ± 12.23	259 ± 15.90	242.16 ± 14.43**	320.83 ± 13.51
Group V	METS (400mg/kg)	297.5 ± 10.38	245.33 ± 14.76	212 ± 18.47	206.83 ± 18.75	166.83 ± 15.99	137.5 ±12.06	126.66 ± 13.01**

Antidiabetic effect of *T. spathacea* extract Values are mean ± SD, n = 6 in each group, values are statistically significant at *P<0.05 and more significant at **P<0.01 when compared with a diabetic control group (alloxan-induced)

Table 3: Effect of *T. spathacea* extract on blood glucose levels (mg /dl) of streptozotocin-induced diabetic rats

Group	Treatment	Blood glucose level mg/dl				
		1st day	3 day	5 thday	7 thday	14th day
Group I	Normal control(Saline)	111.4 ±6.34	113.3 ±7.43	114.5 ±8.84	112± 9.97	165 ± 7.73
Group II	Diabetic Control	284.9 ±93.55	283.9 ±91.25	279.8 ±88.71	283 ± 43.92	281.8 ±37.44
Group III	Glibenclamide	289.4 ±3.8	258.9 ±4.9	163.3± 6.51	137.3 ± 3.96	125.9 ±5.4***
Group IV	METS (200mg/kg)	287.4 ±13.2	263.4 ±18.5	198.2 ±20.8	152.6 ±24.54	133.8 ±29.4*
Group V	METS (400mg/kg)	289 ±66.2	261.4 ± 68.2	187.5 ±78.72	104.5 ±86.1	124.8 ±75.1**

Antidiabetic effect of *T. spathacea* extract Values are mean ± SD, n = 6 in each group, *P<0.05, **P<0.01 when compared with a diabetic control group (streptozotocin-induced)

Table 4: Lipid profile in alloxan-induced diabetic rats

Group	Treatment	Cholesterol (mg/dL)	TGs (mg/dL)	HDL (mg/dL)
Group I	Normal control(Saline)	56.17±2.35	65.17±1.79	41.33±1.02
Group II	Diabetic Control	127.33±2.24	77.67±1.33	30.67±0.95
Group III	Glibenclamide	64.67±1.80	63.17±1.30	40.67±1.20
Group IV	METS (200mg/kg)	120.17±1.97**	75.17±1.4	31.17±0.60**
Group V	METS (400mg/kg)	84±2.03	64.33±1.80**	39.17±0.83**

Antidiabetic effect of *T. spathacea* extract values are mean ± SD, n = 6 in each group, *P<0.05, **P<0.01 when compared with a diabetic control group (alloxan-induced)

Table 5: Lipid profile in streptozotocin-induced diabetic rats

Group	Treatment	Cholesterol (mg/dL)	TGs (mg/dL)	HDL (mg/dL)
Group I	Normal control (Saline)	60.11± 2.13	70.17±1.89	38.33± 2.11
Group II	Diabetic Control	135.36± 1.79	80.69± 2.42	32.72± 1.77
Group III	Glibenclamide	65.53± 1.71	62.66± 1.54	43.66± 1.71
Group IV	METS (200mg/kg)	115.32 ± 1.97*	80.21 ±1.42*	33.21± 1.60*
Group V	METS (400mg/kg)	70±2.112**	63.33± 2.80*	33.44 ± 1.23**

Antidiabetic effect of *T. spathacea* extract Values are mean ± SD, n = 6 in each group, *P<0.05, **P<0.01 when compared with the diabetic control group (streptozotocin-induced)

Table 6: Effect of *T. spathacea* extract on volume, pH of gastric content, total acidity, free acidity in pylorus ligated rats

Treatment Dose (mg/kg)	The volume of gastric juice in ml	pH	Total acidity	Free acidity
Control	5.5±0.428	3.77±0.03	17.9± 0.36	0.19 ±0.007
Ulcerated control	6.18±0.17	1.67±0.05	45.7 ± 1.21	0.25 ±0.009
Ranitidine (30mg/kg)	1.73±0.09	3.50±0.06	6.21± 0.2	0.07 ±0.004
METS (200mg/kg)	4.96± 0.24	2.38±0.07	15.58± 0.4	0.18 ±0.006
METS (400mg/kg)	2.56± 0.18	3.34±0.10	7.21± 0.30	0.10 ±0.004

Values are mean ± SEM for 6 rats.

Table 7: Effect of *T. spathacea* extract on ulcer index and % gastric ulceration in pylorus ligated rats

Treatment Dose (mg/kg)	Ulcer index	% Gastric protection
Control	00.00±0.00	-
Ulcerated control	10.25± 0.89	-
Ranitidine (30mg/kg)	1.66±0.088	83.8
METS (200mg/kg)	5.33± 0.49*	48.0*
METS (400mg/kg)	2.55± 0.20**	75.12**

Values are mean ± SEM for 6 rats. Superscript indicates significant difference at P <0.001** and P <0.05* when compared to the control group

Table 8: Effect of *T. spathacea* extract on volume, pH of gastric content, total acidity, free acidity in ethanol-induced ulcers in rats

Treatment Dose (mg/kg)	The volume of gastric juice in ml	pH	Total acidity	Free acidity
Control	2.23 ± 0.22	1.89±0.12	73.48±3.33	47.30±2.73
Ulcerated control	3.47±0.28	1.26±0.19	101.59±9.56	81.35±2.56
Sucralfate (400 mg/kg)	1.43±0.09	2.84±0.32	39.99±2.96	24.64±1.08
METS (200mg/kg)	2.36±0.10	2.32±0.18	53.34±3.59	30.62±1.77
METS (400mg/kg)	1.82±0.28	2.69±0.20	43.76±2.03	26.45±0.74

Values are mean ± SEM for 6 rats.

Table 9: Effect of *T. spathacea*extract on ulcer index and % gastric protection in methanol induced ulcers in rats

Treatment Dose (mg/kg)	Ulcer index	% Gastric protection
Control	00.00±0.00	-
Ulcerated control	18.5±4.86	-
Sucralfate (400 mg/kg)	1.45±0.32	92.16
METS (200mg/kg)	5.10±1.21**	72.43*
METS (400mg/kg)	2.50±0.99**	86.49**

All values are in mean ± SEM, n=6, VGJ = Volume of gastric juice in ml; TA = Total acidity in mEq/l., FA = Free acidity in mEq/l; UI = Ulcer index, P <0.05* ,P <0.001 = **; The volume of sodium hydroxide required corresponds to the total acidity. Acidity (mEq /l/100g) was calculated as; Acidity = {Volume of NaOH X Normality X 100 mEq/l/100g X 0.1}.

and 5 mg/kg (UI; 2.50±0.99) was found in the ulcer index (tables 7 and 9) in animals pretreated with test extract. In contrast to ulcerated power, it displayed gastroprotection of 72.43 percent at 200 mg/kg and gastroprotection of 86.49 percent at 400 mg/kg.

DISCUSSION

Antidiabetic activity

T. spathacea extract has a remarkable effect on the amount of blood glucose equivalent to the reference drug (table 2). A decline in blood cholesterol, TG, and HDL level was observed. However, the administration of *T. spathacea* methanolic extracts greatly improved the lipid content at doses of both 200mg/kg and 400mg/kg (table: 4 and 5).

Antiulcer activity

Pyloric ligation method

The results suggest that the higher dose of the test extract of 400 mg/kg was effective in protecting ulcers in pylorus-linked rats. In all pretreated animals with 20 mg/kg of Ranitidine, Pylorus ligation formed ulcers. However, the ulcer index (1.66±0.088) showed major

reductions compared to ulcer treatment and gastroprotection was 83.80 percent.

Ethanol-induced ulcers

The results indicate that the higher dose of the test extract, i.e. 400mg/kg, was effective in protecting rat ulcers caused by gastric ulcers caused by ethanol. Ulcers of 400 mg/kg sucralfate were produced by ethanol in all pretreated animals. However, relative to ulcer management, the ulcer index (1.45±0.32) saw substantial declines and gastro safety was 92.16 percent.

CONCLUSION

Niveaus of blood glucose *T. spathacea* extracts treated animals were found to be significantly lower in the oral glucose tolerance test, diabetes-induced alloxan, diabetes-induced models of streptozotocin compared to diabetic control. Antiulcer activity of *T. spathacea* extract results was found significantly. Our findings suggest *T. spathacea* extract has important Antidiabetic properties, as well as antiulcer activity.

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