Evaluation of antidiabetic and antianxiety potential of *Kalanchoe pinnata* root standardized extracts

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Received 28 September 2016
Accepted 15 December 2016

Egyptian Pharmaceutical Journal
2017, 16:24–36

Background
Kalanchoe pinnata is an important medicinal plant and possesses numerous medicinal values including antidiabetic potential.
Aim
The aim of this study was to explore the antidiabetic and antianxiety potential of *K. pinnata* root standardized extracts.
Materials and methods
Standardization of the extracts included the physicochemical and fingerprinting analysis of the extracts. Pharmacological studies included acute toxicity, antianxiety, the oral glucose tolerance test, and streptozotocin (STZ)-induced antidiabetic screening of standardized extracts. Diabetes was induced by a single intraperitoneal injection of STZ (65 mg/kg). Fasting blood glucose (FBG) levels were monitored in normal and diabetic rats at regular intervals of 0, 7, 14, and 21 days of the experiment. The antianxiety potential of ethyl acetate and ethanol extract at the doses of 100, 200, and 300 mg/kg was evaluated using an elevated plus maze model.
Results
Ethyl acetate extract at 300 mg/kg was found to have significant antianxiety potential, whereas ethanol extract at 100, 200, and 300 mg/kg failed to show a statistically significant antianxiety effect. Alcohol and aqueous extracts were found to show a statistically significant ($P<0.001$) decrease in FBG at 400 mg/kg, whereas both extracts at low doses failed to show a significant decrease in FBG on the oral glucose tolerance test. Treatment of STZ-induced diabetic rats with the alcohol and aqueous extract (200 and 400 mg/kg) led to a statistically significant ($P<0.001$) reduction in FBG of the diabetic rats after the 21st day of treatment.
Conclusion
The present study re-established the plant as an effective antidiabetic and antianxiety armament in fighting with the challenge of diabetes mellitus, expected to cover most of Europe and Asia in coming 20 years.

Keywords:
antianxiety, antihyperglycemic, elevated plus maze, fingerprinting analysis, glibenclamide, streptozotocin

Egypt Pharmaceut J 16:24–36

1687-4315

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and other microvascular and macrovascular abnormalities resulting from either insulin resistance or insulin deficiency, or both [1]. However, because of unwanted side effects and other major complications associated with this 'no returned' disease, there is a huge demand for the establishment of new compounds or drugs [2].

India is the largest producer of medicinal herbs and is thus called the botanical garden of the world. Ethnobotanical information reports about 800 plants that have antidiabetic potential [3]. The current research targets to cure diabetes using herbal products.

Recently, extensive research has been carried out to explore the plants and its extracts with respect to their antihyperglycemic potential. Various plants – such as *Croton* macrostachyus, *Acacia nilotica*, *Cyperus rotundus*, etc. – have been proven to possess significant antidiabetic and antihyperlipidemic potential, thus providing an avenue for research in this area [4–12].

Kalanchoe pinnata, commonly known as 'Master Herb' or 'cure for all', is an important plant that is used to treat ailments such as infections, rheumatism, inflammation, and hypertension and for the treatment of kidney stones. This plant has various pharmacological activities such as anthelmintic, wound healing, hepatoprotective, antiallergic, anti-inflammatory, nephroprotective,

 DOI: 10.4103/1687-4315.205830

antimicrobial activity, analgesic, antihypertensive, neuro - pharmacological, and hematological [8,9]. In traditional medicine, *Kalanchoe* spp. have been used to treat ailments such as infections, rheumatism, and inflammation. A strong antitumor-promoting compound bryophillin A was isolated from *K. pinnata*, whereas isolated bryophillin C showed significant insecticidal activities [13,14].

Plant leaves have been proven to have antidiabetic activity, whereas roots have not been tested for antidiabetic and antianxiety activity on streptozotocin (STZ)-induced type 2 diabetic rats to date. Hence, an attempt has been made to explore the antidiabetic and antianxiety potential of *K. pinnata* root extracts. Fingerprinting analysis was also attempted to identify the major phytoconstituents present in the extracts.

Materials and methods

Chemicals

STZ was obtained from Sigma–Aldrich Co. (St. Louis, Missouri, USA). Common solvents and chemicals were purchased from Chemsynth Laboratories Pvt. Ltd., New Delhi, India. All the chemicals used were analytical grade, whereas other biochemical kits were obtained from Span Diagnostics Ltd (New Delhi, India). Other chemicals of analytical grade used in the study were obtained from the Laboratory of Devsthali Vidyapeeth College of Pharmacy.

Plant material

K. pinnata roots were collected in September 2011 from the nursery of Rudrapur, Uttarakhand. The plant was identified and authenticated by the National Botanical Research Institute, Lucknow. The herbarium of the plant was prepared and deposited in the herbarium of Devsthali Vidyapeeth College of Pharmacy (Varsha no. 10).

Preparation of extracts

The shade-dried root powder (100 g) was extracted exhaustively with 70% v/v ethanol in a Soxhlet apparatus by continuous heat extraction. The extract was concentrated to a small volume and then evaporated to dryness. The alcohol extract for experimental purposes was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent; Chemsynth Laboratories Pvt. Ltd., New Delhi, India).

An aqueous extract was prepared by maceration with chloroform water, followed by filtration and concentrating the extract to a small volume and then evaporating to dryness. The aqueous extract for experimental purposes was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent; Chemsynth Laboratories Pvt. Ltd., New Delhi, India).

Microscopic studies

Microscopic studies included examination of transverse sections and determination of powder characteristics of the plant to establish the diagnostic characteristics of the plant [15,16].

Physicochemical studies

This included the evaluation of physicochemical parameters such as moisture content, ash value, extractive value, and fluorescence studies.

Preliminary phytochemical studies

Phytochemical studies included the detection of major class phytoconstituents and then selection of mobile phases for the chromatographic analysis [17,18].

Chromatographic studies

Thin-layer chromatography (TLC) of extracts was performed in different mobile phases to select the best mobile phases for the major class of phytoconstituents present in the extracts. Fingerprinting analysis was carried out in the best chosen mobile phases in four mobile phases for the detection of different phytoconstituents:

Mobile phase I: $CHCl_3$: C_2H_5OH (9.8 : 0.2).

Mobile phase II: $CHCl_3$: CH_3OH (95 : 5).

Mobile phase III: toluene : ethyl acetate : glacial acetic acid (5 : 7 : 1).

Mobile phase IV: toluene : ethyl acetate : glacial acetic acid (6 : 4 : 1).

Pharmacological activities

Experimental animals

For the experiment, male Wistar rats weighing between 200 and 230 g were obtained from the Animal Research Branch of the Indian Veterinary Research Institute (Bareilly, Uttar Pradesh, India). Swiss albino mice weighing 20–25 g of either sex were used for the acute toxicity studies. Animals were maintained under standard environmental conditions (temperature: 24.0±1.0°C, relative humidity: 55–65%, and 12 h light/12 h dark cycle), and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before the experiments. All protocols of the animal experiments were approved by the institutional animal ethical committee (CPCSEA/IAEC/2010-11/06).

Acute toxicity studies

Acute toxicity studies were carried out to evaluate the toxic profile and to determine the minimum lethal dose of the extracts. Swiss albino mice of either sex weighing between 20 and 25 g fasted overnight were used for the study. The extracts were administered orally at doses of 30, 100, 300, 500, 1000, and 3000 mg/kg body weight. Subsequent to the administration of drug extracts, animals were observed closely for the first 3 h for any toxic manifestations (increased motor activity, sedation, acute convulsion, coma, and death). Thereafter, the observations were made at regular intervals for 24 h. The animals were kept under further observation for 1 week [19,20].

Antianxiety activity

The antianxiety potential of ethanol and ethyl acetate extracts was evaluated at four different doses of 100, 200, 300, and 400 mg/kg, and diazepam was used as the standard drug. The elevated plus maze (EPM) model of anxiety was used for the assessment of antianxiety potential. Each mouse was placed at the center of an EPM with its head facing the open arms. The behavior of each mouse was recorded during the experiment as follows:

(1) The number of entries in the open arms.
(2) Average time spent by the mouse in the open arms (average time=total spent in open arms/number of entries in arms).

Plant extracts were administered orally using a tuberculin syringe fitted with an oral cannula. The dose administration schedule was adjusted so that each mouse had its turn on the EPM apparatus for 45 min after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of plus-maze, could induce anxiety in the animals [21].

Antidiabetic activity

Oral glucose tolerance test

Overnight-fasted rats were divided into six groups of six animals each.

Experimental protocol:

(1) Group I was used as a control, and received 5% Tween 80.
(2) Group II was the standard group.
(3) Group III received an alcohol extract (200 mg/kg).
(4) Group IV received an alcohol extract (400 mg/kg).
(5) Group V received an aqueous extract (200 mg/kg).
(6) Group VI received an aqueous extract (400 mg/kg).

Animals of groups II, III, IV, V, and VI were loaded with glucose (2 g/kg orally) after 30 min of drug administration. Blood samples were collected by puncturing the retro-orbital sinus just before drug administration and at 30, 60, 90, and 120 min after loading glucose. Serum glucose level was measured immediately using a glucose estimation kit (Span Diagnostics Pvt Ltd) [22].

Induction of diabetes in rats

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (65 mg/kg) in 0.1 mol/l citrate buffer (pH 4.5) to overnight-fasted rats. After 48 h of STZ administration, blood was withdrawn from the retro-orbital plexus and plasma was separated by centrifugation. Fasting blood glucose (FBG) levels were measured immediately using the glucose oxidase/peroxidase method to confirm the presence of diabetes.

Estimation

Blood glucose level (BGL), total cholesterol (TC), high-density lipoprotein cholesterol, and triglycerides were estimated using standard kits of Span Diagnostic Ltd.

Experimental design

Assessment of antidiabetic activity in streptozotocin-induced diabetic rats:

Diabetic rats were divided into seven groups of six animals each for the assessment of antidiabetic activity.

(1) Group I: normal control, which received food and water.
(2) Group II: diabetic control, which received 0.5 ml of 5% Tween 80.
(3) Group III: standard, which received glibenclamide (100 μg/kg).
(4) Groups IV and V: aqueous extract-treated group (200 and 400 mg/kg).
(5) Groups VI and VII: alcohol extract-treated group (200 and 400 mg/kg).

The treatment was continued daily for 21 days. Blood samples were collected from the retro orbital sinus of overnight-fasted animals just before drug administration for glucose estimation on days 0, 7, 14, and 21 of the experiment [23,24].

Statistical analysis

The data were expressed as mean±SD. Statistical comparisons were performed by two-way analysis of variance (ANOVA), followed by Duncan's multiple range test. The results were considered statistically significant if the *P* values were 0.05 or less.

Fingerprinting analysis

Chromatographic studies were carried out [25,26]. In the present work, a Camag high-performance thin-layer chromatography (HPTLC) system (Switzerland) equipped with a Linomat V applicator, a TLC scanner 3, and Reprostar 3 with a 12-bit charge coupled device camera for photo documentation, controlled by Win CATS-4 software, was used. All the solvents used were HPTLC grade obtained from Merck. All weighings were performed on a Precisa XB 12A digital balance.

Preparation of extract

The alcohol extract for analysis was prepared by dissolving the extract in alcohol, followed by sonication. The aqueous extract was prepared by dissolving the extract, followed by sonication and centrifugation; the supernatant was used for sampling.

Mobile phases

The following mobile phases were used:

Mobile phase I: $CHCl_3$: C_2H_5OH (9.8 : 0.2).

Mobile phase II: $CHCl_3$: CH_3OH (95 :5).

Mobile phase III: toluene : ethyl acetate : glacial acetic acid (5 : 7 : 1).

Mobile phase IV: toluene : ethyl acetate : glacial acetic acid (6 : 4 : 1).

Chamber used for the mobile phase

Camag twin trough chamber (10×10 cm) was used for the mobile phase.

Chamber saturation

Chamber saturation was performed for 18 h.

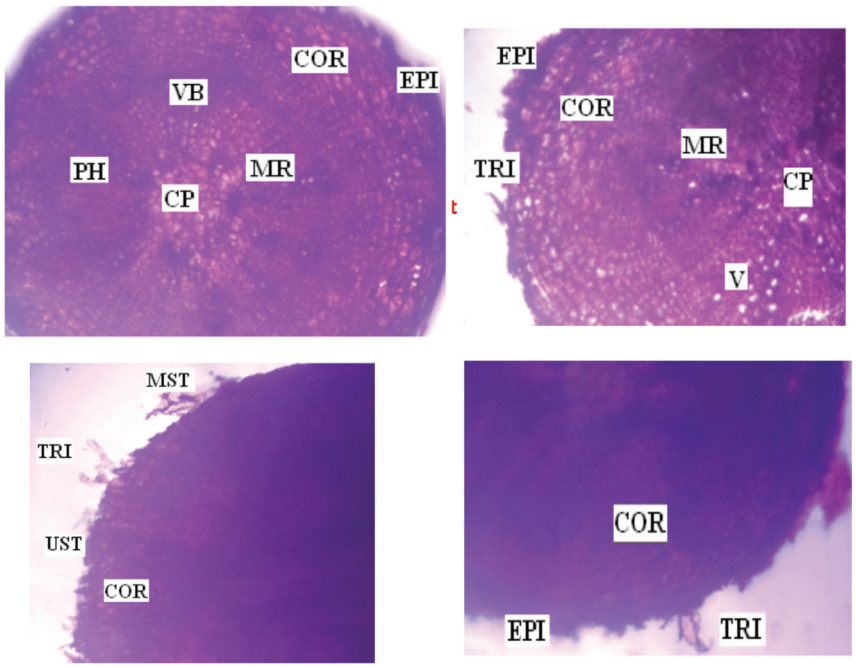
Stationary phase

HPTLC plates of silica gel 60 F254 manufactured by E. Merck KGaA (5×10 cm) were used as the stationary phase.

Procedure

The samples of aqueous and alcohol extracts were prepared according to the method described above. The TLC plates were activated by heating at 120°C for about 30 min before use. Alcohol extract (2 µl) and aqueous extract (2 µl) were each applied in duplicate, as tracks 1–6, with a band length of 8 mm each on a precoated silica gel 60 F254 TLC plate, with a Linomat V applicator using a Hamilton syringe. No prewashing of the plate was performed. Chamber

Figure 1



Section showing the different types of trichomes present in *K.pinnata* root section

Section showing the different portions of *K. pinnata* root. COR, cortex; CP, central pith; EPI, epidermis; MR, medullary rays; MST, multiseriate trichomes; OC, outer cortex; PH, phloem; TRI, trichomes; UST, uniseriate trichomes, V, vessels; VB, vascular bundle; XY, xylem.

saturation time was 18 h. The TLC plate was kept for development to a migration distance of 77 mm and scanned at 254 and 366 nm, band length 8 mm, slit dimension, and scanning speed, and the source of radiation was deuterium and tungsten lamps, respectively. The developed plates were dried and scanned successively at wavelengths of 254, 366, and 425 nm, band width, slit dimension, scanning speed and the source of radiation was deuterium, tungsten and mercury. The R_f and peak area of the spots were interpreted using software [27].

Results and discussion

Pharmacognostical studies

Macroscopical characteristics

Roots collected from the nursery were found to be dark brown in color, odorless, bitter in taste, and varying in size. It contained bark, which was easily separable and with fibrous fractures. The root was found to be thin and easily breakable.

Table 1 Treatment of root sections with different reagents

Drugs	Test for	Reagent	Reaction	Result
Section	Lignin	Phloroglucinol +diluted HCl	Red color	+
Section	Mucilage	Ruthenium red solution	No red color	–
Section	Starch	Iodine solution	Blue color	++
Section	Fixed oil	Sudan red III solution	No red color	–
Section	Crystals	Concentrated HCl	Effervescence	++

+, present; –, absent.

Microscopical characteristics

Transverse section of the root showed the presence of the characteristics presented in Fig. 1.

The presence of trichomes (uniseriate and multiseriate trichomes) was noted. Transverse section showed the specific characteristics of dicot plants. The section showed the secondary growth of root. The presence of glandular trichomes, vascular bundles, primary and secondary xylem, secondary phloem, medullary rays, and central pith was observed. It was also found that a number of starch grains had spread across all sections.

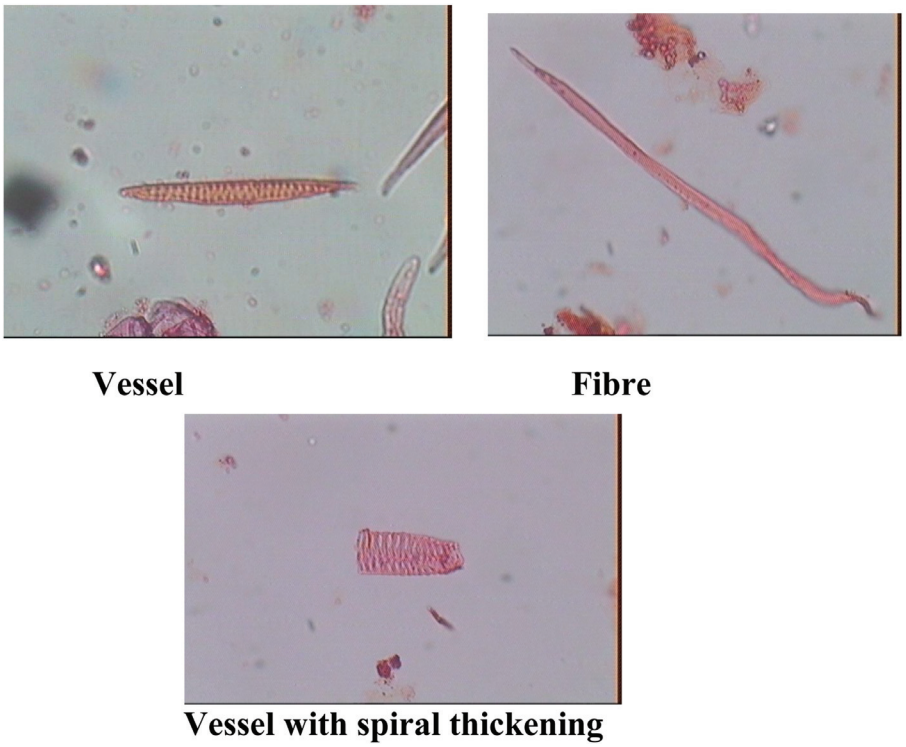
Histochemical studies

The results of histochemical studies are shown in Table 1.

Powder characteristics

Fragments of trichomes, uniseriate, multiseriate trichome, parenchymatous cells, groups of cork cells, vessels with spiral and reticulate thickening, fragments of various types of fibers, and tracheids were also observed as shown in Fig. 2.

Figure 2



Powder characteristics of the *Kalanchoe pinnata* root.

Physicochemical constants

The results are presented in Tables 2–6.

Fingerprinting analysis of alcohol and aqueous extracts

Fingerprinting analysis of alcohol extract (2 µl) showed the presence of seven prominent peaks with R_f 0.02, 0.10, 0.11, 0.31, 0.52, 0.66, and 0.74 in mobile phase I; five prominent peaks at R_f 0.01, 0.15, 0.61, 0.67, and 0.78 in mobile phase II; three prominent peaks at R_f 0.02, 0.61, and 0.67 in mobile phase III; and six prominent peaks at R_f 0.02, 0.07, 0.20, 0.27, 0.72, and 0.83 in mobile phase IV as shown in Tables 7–10 and Figs 3–6.

Among the above prominent peaks, the peak at R_f 0.42 belonged to 7-hydroxy flavones and the peak at R_f at 0.14, 0.6, and 0.27 belonged to morin and 6-hydroxy flavones and quercetin, respectively [28].

The results showed that the alcohol extract was found to be rich in flavonoids, glycosides, and phenolic compounds, and found to lack cardiac glycosides. The alcohol extract showed a prominent spot at R_f 0.02, 0.06, 0.14, 0.24, 0.36, 0.50, 0.61, 0.05, and 0.35 quenched fluorescence at 254 and 366 nm, respectively. All spots quenched fluorescence at 254 nm, and showed blue and violet fluorescence at 366 nm.

Fingerprinting analysis of the aqueous extract (2 µl) showed the presence of eight prominent peaks at R_f 0.03, 0.05, 0.10, 0.13, 0.31, 0.50, 0.68, and 0.74 in mobile phase I (flavonoids); four prominent peaks at R_f 0.51, 0.65, 0.70, and 0.77 in mobile phase II (glycosides); six prominent peaks at R_f 0.01, 0.04, 0.12, 0.23, 0.60, and 0.68 in mobile phase III (phenolic compounds); and three prominent peaks at R_f 0.02, 0.08, 0.18, 0.31, 0.36, 0.73, and 0.84 in mobile phase IV (phenolic compounds), respectively as shown in Tables 11–14 and Figs 7–10. Among the various peaks showed above the peak with R_f 0.60 belongs to the Chrysin in aqueous extracts [29].

The results showed that the aqueous extract was found to be rich in flavonoids and phenolic compounds. The aqueous extract showed a prominent spot at R_f 0.09, 0.17, 0.23, 0.60, 0.68, 0.73, 0.77, 0.86, and quenched fluorescence at 254 and 366 nm, respectively. All spots quenched fluorescence at 254 nm and showed blue and violet fluorescence at 366 nm. Photographs of HPTLC plates are shown in Figs 11 and 12.

Table 2 Moisture content of *Kalanchoe pinnata* root

Fresh weight (g)	Dry weight (g)	Loss on drying (g)	Moisture content (%)
2.00	1.42	0.58	9.25

Table 3 Ash values of *Kalanchoe pinnata* root

Total ash (%)	Acid-insoluble ash (%)	Water-soluble ash (%)
8.92	1.86	3.58

Table 4 Extractive values of *Kalanchoe pinnata* root

Extractives	Extractive values (% w/w)
Alcohol soluble	6.21
Water soluble	9.47

Table 5 Successive solvent extractive values and nature of *Kalanchoe pinnata* root extracts

Solvents	Color	Consistency	Extractive value (% w/w)
Petroleum ether	Blackish brown	Semisolid sticky	0.65
Benzene	Brown	Sticky mass	1.35
Chloroform	Chocolate brown	Sticky mass	1.16
Acetone	Black	Sticky mass	2.31
Alcohol	Dark brown	Sticky mass	2.58
Aqueous	Yellowish brown	Sticky mass	4.87

Pharmacological studies

Acute toxicity studies

Acute toxicity studies showed that ethanol and aqueous extracts were found to be safe up to doses of 3000 mg/kg in albino mice.

Table 6 Preliminary phytochemical analysis of *Kalanchoe pinnata* root extracts

Test for	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Aqueous
Alkaloids	–	–	–	–	+	++
Carbohydrates and glycosides	–	–	+	–	+	++
Phytosterols	+	+	+	+	–	–
Fixed oils and fats		+	+	–	+	++
Phenolic compounds and tannins	–	–	–	+	+	+
Saponins	–	–	–	–	–	++
Flavonoids	–	–	–	–	+	+++
Gums and mucilage	–	–	–	–	–	–
Volatile oils	–	–	–	–	–	–

+, present; –, absent.

Table 7 Peak table of *Kalanchoe pinnata* alcohol extracts in mobile phase IV

Peaks	R_f	Height	Area	Assigned substance
1	0.02	402.8	3373.0	Unknown
2	0.07	31.8	661.3	Unknown
3	0.20	57.2	1469.3	Unknown
4	0.27	80.6	2639.6	Quercetin[a]
5	0.72	679.5	63056.1	Unknown
6	0.83	16.2	202.1	Unknown

[a]Fingerprinting analysis and the marked constituents are identified on the basis of R_f.

Table 8 Peak table of *Kalanchoe pinnata* alcohol extracts in mobile phase III

Peaks	R_f	Height	Area	Assigned substance
1	0.02	634.0	6664.7	Unknown
2	0.61	38.8	1152.2	6-Hydroxy flavone[a]
3	0.67	44.4	1415.7	Unknown

[a]Fingerprinting analysis and the marked constituents are identified on the basis of R_f.

Table 9 Peak table of *Kalanchoe pinnata* alcohol extracts in mobile phase II

Peaks	R_f	Height	Area	Assigned substance
1	0.01	86.6	833.7	Unknown
2	0.15	11.7	139.6	Unknown
3	0.61	18.7	212.0	Unknown
4	0.67	15.5	107.7	Unknown
5	0.78	19.8	583.5	Unknown

Table 10 High-performance thin-layer chromatography chromatogram of *Kalanchoe pinnata* alcohol extracts in mobile phase I

Peak	R_f	Height	Area	Assigned substance
1	0.10	15.7	289.3	Unknown
2	0.11	18.6	173.8	Unknown
3	0.31	18.0	485.8	Unknown
4	0.52	11.9	345.4	Unknown
5	0.66	33.4	1134.2	Unknown
6	0.74	110.2	4167.3	Unknown

Antianxiety studies

Analysis of extracts was carried out on ethanol and ethyl acetate extracts at 100, 200, 300, and 400 mg/kg body weight. Among the doses, the ethyl acetate extract showed significant increases in open arm entries and the mean time spent in open arms at the dose of 300 mg/kg. Two-way ANOVA analysis indicated that plant ethyl acetate extract showed a statistically significant antianxiety potential at the dose of 300 mg/kg body weight.

Ethanol extract all the tested doses (100, 200, 300, and 400 mg/kg) failed to show a statistically significant antianxiety effect as shown in Fig. 12.

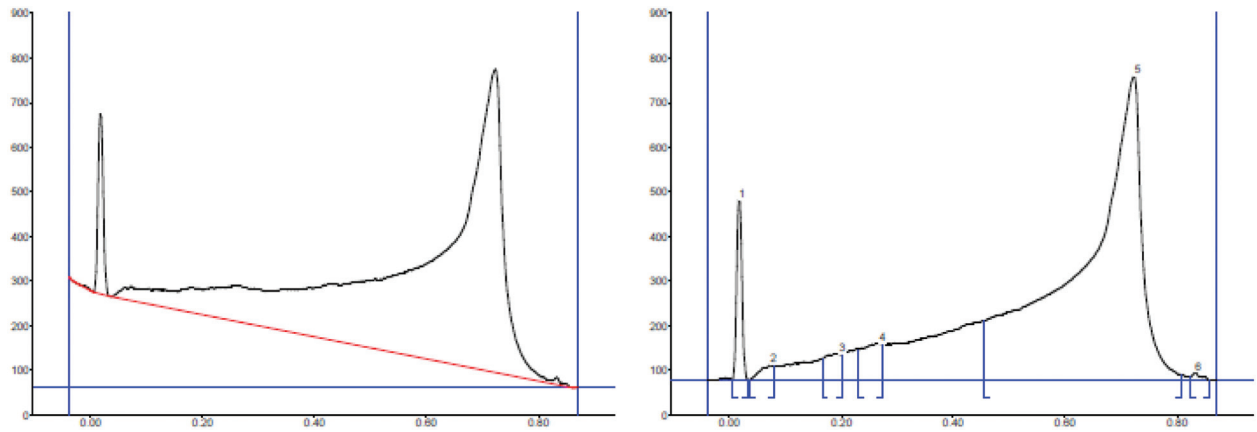
Oral glucose tolerance test

Alcohol and aqueous extracts at both the doses (200 and 400 mg/kg) failed to lower the elevated FBG even after 90 min of extract administration. Aqueous extract at a high dose reduced the FBG after 120 min of extract administration and was found to show a mild but statistically insignificant effect. The results of glucose tolerance test of alcohol and aqueous extracts are shown in Table 15 and Fig. 13.

Antidiabetic activity of K. pinnata root alcohol (KPRAL) and K. pinnata root aqueous (KPRAQ) extract

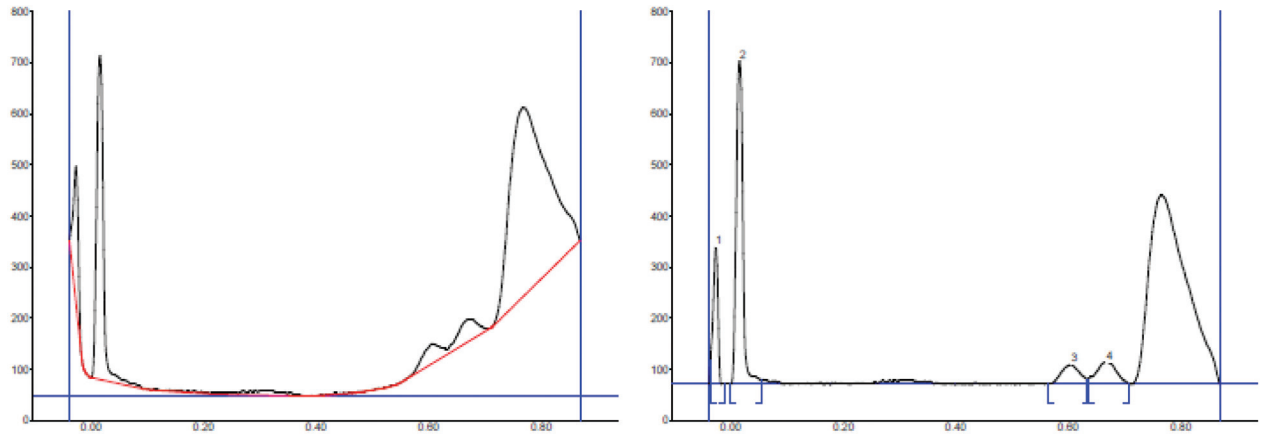
In normal control rats, FBG levels were determined at 0, 7, 14, and 21 days after treatment with vehicle, and were almost similar to the pretreatment levels. In diabetic control rats, the FBG increased gradually up to 21 days. The results of the antidiabetic activity of alcohol and aqueous extracts are shown in Table 16 and Fig. 14. However, administration of extracts at doses of 200 and 400 mg/kg of the extract showed a marked antihyperglycemic effect in diabetic treated rats. On comparing the extracts, alcohol and aqueous extracts at a high dose (i.e. 400 mg/kg), were found to show a

Figure 3



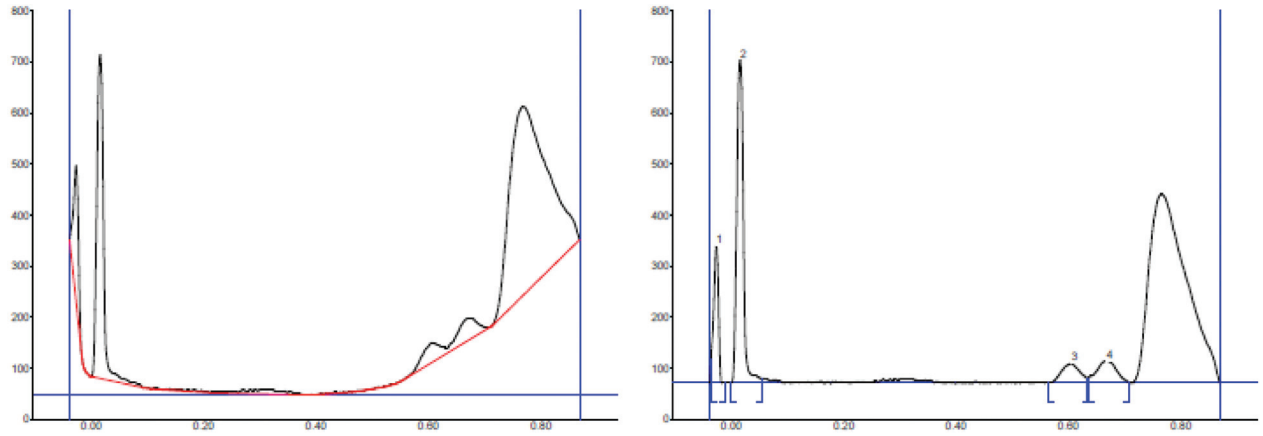
High-performance thin-layer chromatography chromatogram of KPRAL in mobile phase IV.

Figure 4



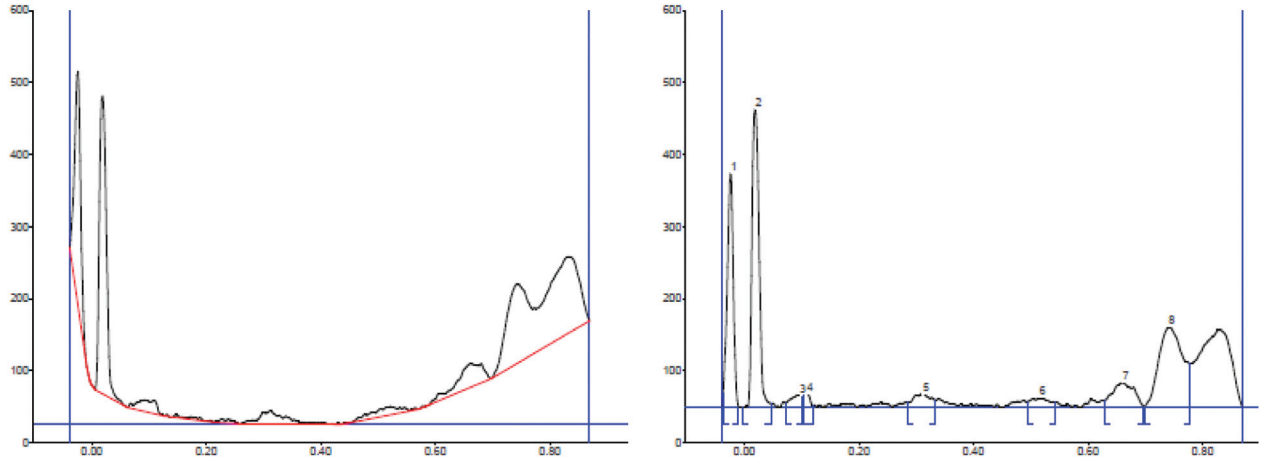
High-performance thin-layer chromatography chromatogram of KPRAL in mobile phase III.

Figure 5



High-performance thin-layer chromatography chromatogram of KPRAL in mobile phase II.

Figure 6



High-performance thin-layer chromatography chromatogram of KPRAL in mobile phase I.

statistically significant antihyperglycemic activity. Animals were observed for food and water intake, and change in body weight during the experiment.

In the present study, *K. pinnata* was selected for the evaluation of antihyperglycemic and antianxiety effects owing to its traditional uses. This study was carried out

Table 11 High-performance thin-layer chromatography chromatogram *Kalanchoe pinnata* aqueous extracts in mobile phase IV

Peak	R_f	Height	Area	Assigned substance
1	0.02	88	751.1	Unknown
2	0.08	52.3	1256.2	Unknown
3	0.18	61.6	2566.6	Unknown
4	**0.31**	86.2	3198.3	Caffeic acid[a]
5	0.36	91.9	2357.9	Unknown
6	0.73	662.9	53111.6	Unknown
7	0.84	17.0	75.9	Unknown

[a]Fingerprinting analysis and the marked constituents are identified on the basis of R_f. Bold values are identified as well as the important constituents may be responsible for the marked observed activities.

Table 13 High-performance thin-layer chromatography chromatogram of *Kalanchoe pinnata* aqueous extracts in mobile phase II

Peaks	R_f	Height	Area	Assigned substance
1	0.51	17.1	521.0	Unknown
2	0.65	18.9	243.3	Unknown
3	0.70	11.2	183.2	Unknown
4	0.77	25.7	715.5	Unknown

Table 12 High-performance thin-layer chromatography chromatogram of *Kalanchoe pinnata* aqueous extracts in mobile phase III

Peaks	R_f	Height	Area	Assigned substance
1	0.04	56.9	793.8	Unknown
2	0.12	19.0	326.8	Unknown
3	0.23	35.7	1354.3	Unknown
4	0.60	17.4	532.6	Chrysin[a]
5	0.68	42.6	1330	Unknown

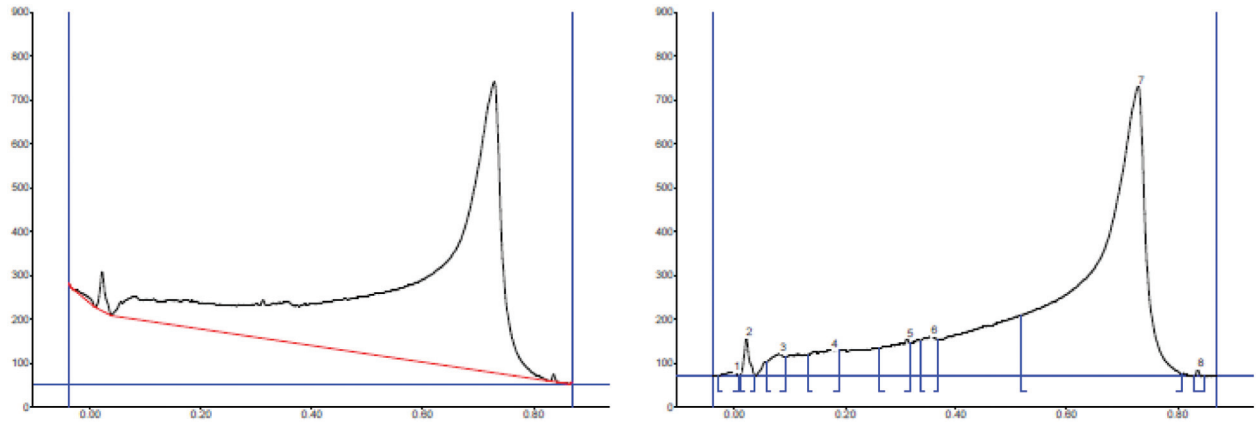
[a]Fingerprinting analysis and the marked constituents are identified on the basis of R_f.

Table 14 High-performance thin-layer chromatography chromatogram of *Kalanchoe pinnata* aqueous extracts in mobile phase I

Peaks	R_f	Height	Area	Assigned substance
1	0.05	23.0	299.1	Unknown
2	0.10	23.1	342.9	Unknown
3	0.13	23.2	450.9	Unknown
4	0.31	14.0	222.7	Unknown
5	**0.50**	11.8	222.6	Marmesin[a]
6	0.68	27.9	956.9	Unknown
7	0.74	56.8	1577.0	Unknown

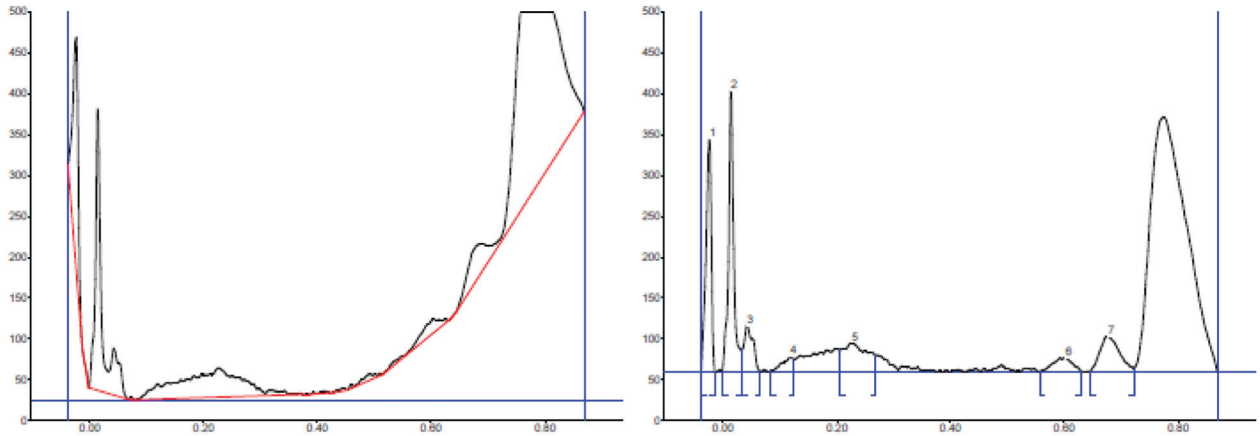
[a]Fingerprinting analysis and the marked constituents are identified on the basis of R_f. Bold values are identified as well as the important constituents may be responsible for the marked observed activities.

Figure 7



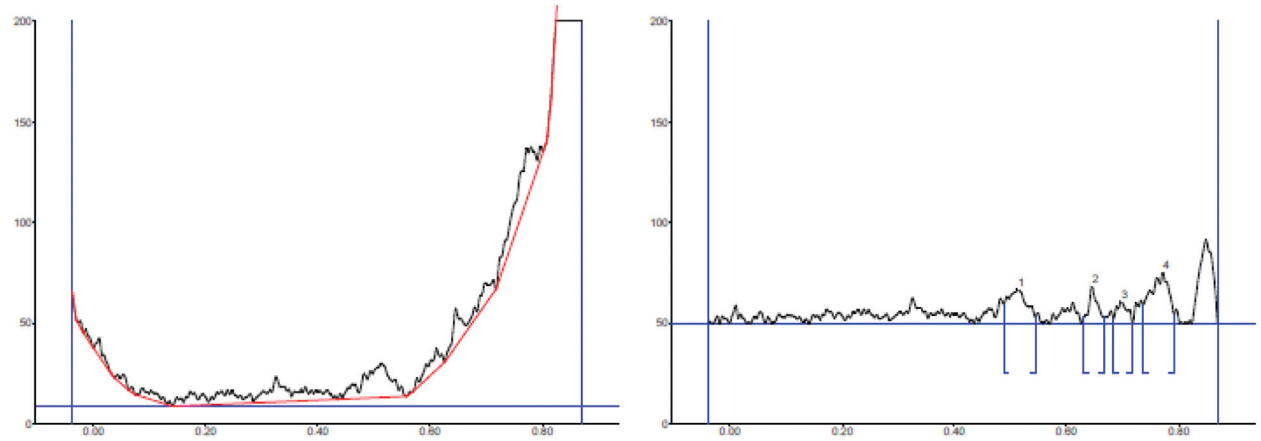
High-performance thin-layer chromatography chromatogram of KPRAQ in mobile phase IV.

Figure 8



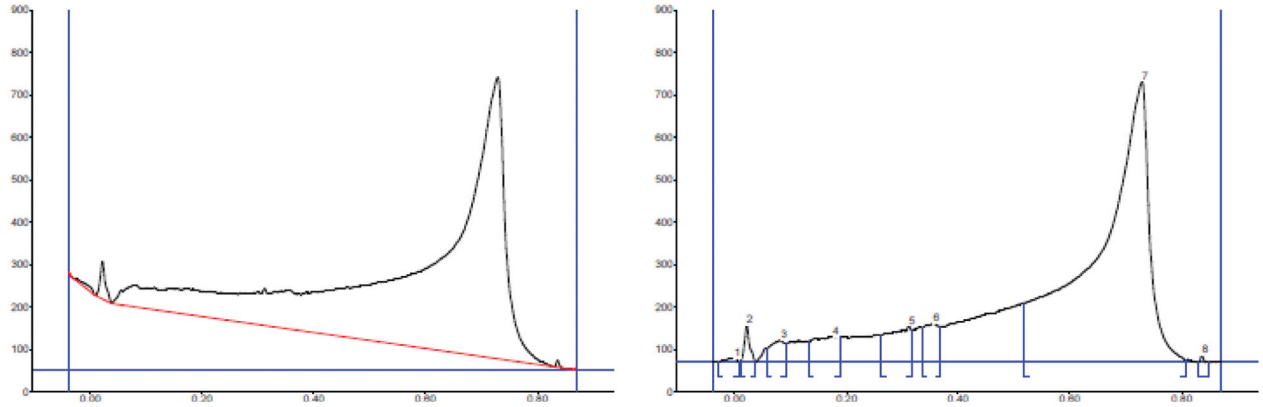
High-performance thin-layer chromatography chromatogram of KPRAQ in mobile phase III.

Figure 9



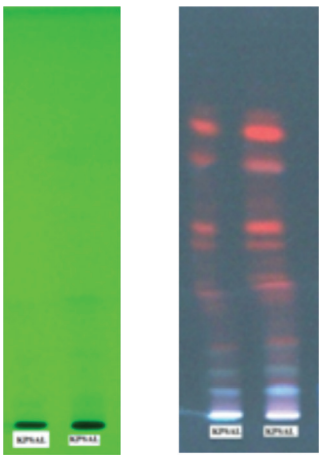
High-performance thin-layer chromatography chromatogram of KPRAQ in mobile phase II.

Figure 10



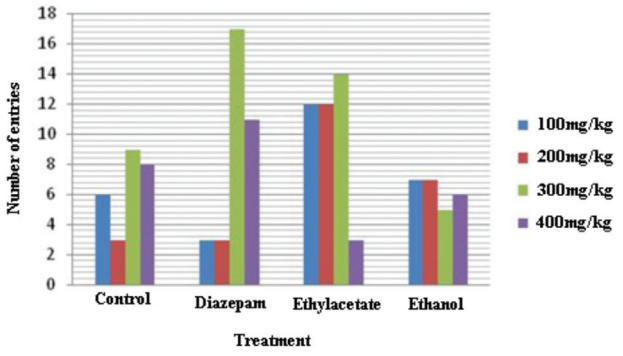
High-performance thin-layer chromatography chromatogram of KPRAQ in mobile phase I.

Figure 11



High-performance thin-layer chromatography plates of KPRAL, KPRAQ at 254 nm in mobile phase-I.

Figure 12



High-performance thin-layer chromatography plates of KPRAL and KPRAQ at 254 and 366 nm. Values are expressed as mean±SEM (n=6). *P<0.05; **P<0.01 compared with initial level of number of entries in open arm of control and treatment groups.

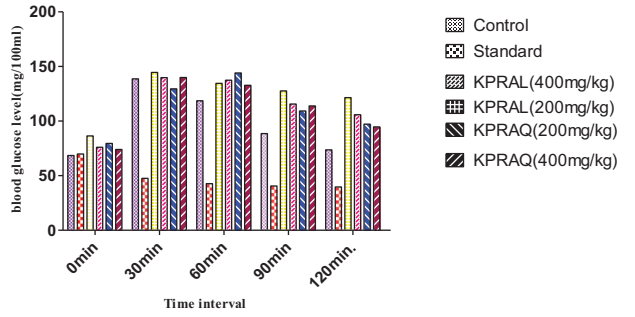
to justify its claimed therapeutic uses. Alcohol and aqueous extracts showed a statistically significant antihyperglycemic potential at the dose of 400 mg/kg. This was further evidenced by statistically significant reductions in BGLs after the 14th and 21st day of oral extract administration of both the extracts.

Table 15 Results of glucose tolerance test of *Kalanchoe pinnata* alcohol and aqueous extracts in normal rats

Groups	Treatments (n=6) (oral)	Blood glucose level (mg/100 ml) (mean±SEM)				
		0 min	30 min	60 min	90 min	120 min
I	Control	68.5±0.61	138.67±0.66	118.6±0.94	88.54±1.02	73.6±0.87
II	Standard	69.86±0.38	47.54±0.54**	42.67±0.32**	40.54±0.9**	39.68±1.94**
III	KPRAL (200 mg/kg)	86.37±5.56	144.60±1.18	134.53±3.72	127.58±2.23	121.53±1.16
IV	KPRAL (400 mg/kg)	76.0±3.52	139.83±7.14	137.46±4.75	115.62±1.12	105.77±6.89
V	KPRAQ (200 mg/kg)	79.52±7.24	129.54±3.32	144.0±1.16	109.17±3.37	97.17±9.67
VI	KPRAQ (400 mg/kg)	74.0±5.62	139.85±1.68	132.79±5.27	113.81±7.39	94.67±10.57

Values are expressed in mean±SEM (n=6). *P<0.05; **P<0.01 compared with the initial level of blood glucose of the rats in the respective groups.

Figure 13



Antianxiety potential of *Kalanchoe pinnata* root extracts. Values are expressed as mean±SEM (n=6). *P<0.05; **P<0.01 compared with initial level of blood glucose of the rats in the respective groups.

Both the extracts failed to show significant oral glucose tolerance test activity, but were found to have statistically significant antidiabetic potential; it seems that the extract may have a mechanism of action that is different from that of conventional oral hypoglycemic agents.

Earlier studies on blueberry extracts with parallel bioactive compounds have also shown reduced food ingestion and body weight in lab animals because of their increased satiating property. However, the exact underlying mechanism was not explained. It has also been shown that blueberries exerted antioxidant effects by decreasing fat accumulation in the aortas and livers of animal models and minimizing the cardiovascular risks in obese individuals [30–33]. These observed effects were believed to be exerted by phytosterols, phenolics, and additional bioactive components present in blueberries as *K. pinnata* extracts [34,35].

As the *K. pinnata* extracts are known to contain flavonoids, polyphenols, triterpenoids, and phytosterols, it is usually believed that these phytoconstituents may be responsible for the hypoglycemic activity of this plant [36]. Reference [37] also showed that the plant might be beneficial in the treatment of diabetes because of the plant's rich source of zinc. An earlier report showed that pharmacological doses of zinc supplementation of animals and humans ameliorate glycemic control in diabetes [38]. Zinc is believed to exert insulin-like effects by supporting the signal transduction of insulin and by reducing the production of cytokines, which lead to β-cell death during the inflammatory process in pancreatic disease [39]. The observed decrease in serum and TC level in the treated diabetic group may also be because of the chemical constituents of the *K. pinnata* preparation. For example, it has been suggested that the ability of phytosterols to lower serum TC is because of the similarity in their chemical structures. They displace intestinal cholesterol with bile salt micelles and compete for absorption from the border brush, thus suppressing the absorption of dietary and biliary cholesterol and upregulation of the enterocyte ATP-binding cassette transport proteins [40,41]. Similarly reference [42], showed that feeding 0.72% phytosterol-containing milk powder decreased TC by 31% and low-density lipoprotein cholesterol by 52% in hypercholesterolemic hamsters. Also, flavone, a class of flavonoids, has been reported to have a lipid-lowering action in hyperlipidemic rats [43]. Reference [44] suggested that flavones may be one of the candidates for an active component in *Zanthoxylum piperitum* extract as in *K. pinnata* aqueous preparations. In their study, sterol-regulatory element binding protein was regulated by flavones, which led to downregulation of transcription factors and fatty acid synthase. They suggested that the inhibitory effect of a *Z. piperitum* extract on high-fat diet-induced obesity may be partially attributed to flavones, which could also explain the decrease in body weight in our study. In addition, several systemic peptides have been shown to regulate appetite and body weight. We suggest further studies on the potential role of *K. pinnata* aqueous and alcohol preparation on vital systemic peptides involved in the regulation of appetite and body weight. Previous studies of various natural extracts containing polyphenols were shown to decrease lipid accumulation and stimulate lipolysis in preadipocytes and adipocytes [45].

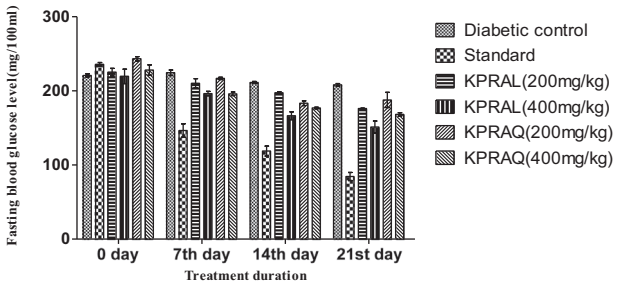
Antianxiety analysis of extracts was carried out on ethanol and ethyl acetate extracts. Two-way ANOVA analysis showed that plant ethyl acetate extract had statistically significant antianxiety

Table 16 Antihyperglycemic activity of alcohol and aqueous extracts in diabetic rats

Groups	Fasting glucose level (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Normal control	76.16±5.36	75.66±3.94	75.0±4.96	80.0±4.61
Diabetic control	220.83±1.92	224.5±3.69	211.3±1.30	208.16±1.74
Standard	235.8±2.38	146.6±9.01**	118.0±6.73***	84.5±5.46***
KPRAL 200 mg/kg	225.20±5.23	210.0±6.14	197.51±1.35*	176.31±0.35**
KPRAL 400 mg/kg	219.56±9.65	196.42±2.83	166.52±5.25*	151.30±7.94**
KPRAQ 200 mg/kg	243.30±2.84	216.79±1.46	183.41±3.21*	187.78±10.32**
KPRAQ 400 mg/kg	228.10±6.98	196.05±2.31	176.9±1.06*	168.31±2.25**

Values are expressed in mean±SEM (n=6). *P<0.05; **P<0.01; ***P<0.001 compared with the initial level of blood glucose of the rats in the respective groups.

Figure 14



Results of glucose tolerance test of KPRAL and KPRAQ extracts in normal rats. Values are expressed as mean±SEM (n=6). *P<0.05; **P<0.01 compared with initial level of blood glucose of the rats in the respective groups.

potential comparable with that of the standard drug diazepam, whereas the ethanol extract failed to show an antianxiety effect as discussed in the results.

The alcohol extract was found to be rich in flavonoids and phenolic constituents, whereas the aqueous extract was found to be rich in flavonoids, phenolic constituents, and glycosides. Fingerprinting analysis showed the presence of 7-hydroxy flavones, morin and 6-hydroxy flavones, and quercetin, respectively, in alcohol extract and chrysin in the aqueous extract. The presence of these important phytoconstituents shows that the extracts may also be effective in the treatment of macrovascular and microvascular complications including the risk of heart attacks, especially in diabetic patients.

Conclusion

From this study, we can conclusively state that alcohol and aqueous extracts of *K. pinnata* root were found to exert remarkable effects on BGLs and lead to a marked improvement in body weight. Therefore, these extracts may be one of the most promising therapeutic armamentarium for the treatment as well as the macrovascular and microvascular complications of diabetes.

Further studies should be carried out at the cellular and molecular levels, which may further elucidate its mechanism in detail. The present investigation has also opened an avenue for further research, especially with reference to the development of potent formulations for diabetes mellitus from *K. pinnata*.

Acknowledgements

The authors are grateful to the National Botanical Research Institute (Lucknow, India) for identification and authentication of the plant specimen. The authors are also grateful to Prof. Edwin Jarald (B. R. Nahata College of Pharmacy, Mandsaur) for providing HPTLC chromatographs and the management of Devsthali Vidyapeeth College of Pharmacy for providing the necessary facilities for the research.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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