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Elucidation of possible mechanisms of the antidiabetic potential of Zn-loaded *Bryophyllum pinnatum* (Lam.) extracts in experimental animal models

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Abstract

Background: The present study focused on the antidiabetic potential and fingerprinting analysis of *Bryophyllumc pinnatum* stems as well as its possible mechanisms of action along with identification of its major phytoconstituents. The oral glucose tolerance test has been performed administering a glucose solution (2000 mg/kg) to induce hyperglycemia. Diabetes have been instigated by single intra-peritoneal injection of streptozotocin (65 mg/kg).

Results: Alcohol and aqueous extracts were found to be safe up to a dose of 3000 mg/kg. Oral glucose tolerance test results showed significant reduction in fasting blood glucose level. Alcohol and aqueous extracts (200, 400 mg/kg b.w.) showed significant reduction in fasting blood glucose among all groups. Groups receiving zinc sulfate-loaded extracts showed a statistically significant reduction in low-density lipoproteins, triglycerides and total cholesterol levels and enhanced levels of high density lipoproteins. Fingerprinting analysis has been performed to identify the major phytoconstituents of flavonoid category morin, chrysin, and 6-hydroxy flavones, as well as iso-quercetin, hyperosides and terpenoids present in the extracts possess antidiabetic potential.

Conclusions: Both alcohol and aqueous extracts found to possess significant antidiabetic activity in diabetic rats. Zinc sulfate synergistically potentiates the antidiabetic potential of alcohol extract. Fingerprinting analysis revealed the presence of flavonoids such as morin, chrysin, and 6-hydroxy flavones, as well as iso-quercetin, hyperosides, and terpenoids. The possible mechanisms of antidiabetic activity have been elucidated, although further studies are required to give more elaborated mechanism on molecular basis.

Keywords: Fingerprinting, OGTT, Streptozotocin, Fasting blood glucose level, Anti-diabetic

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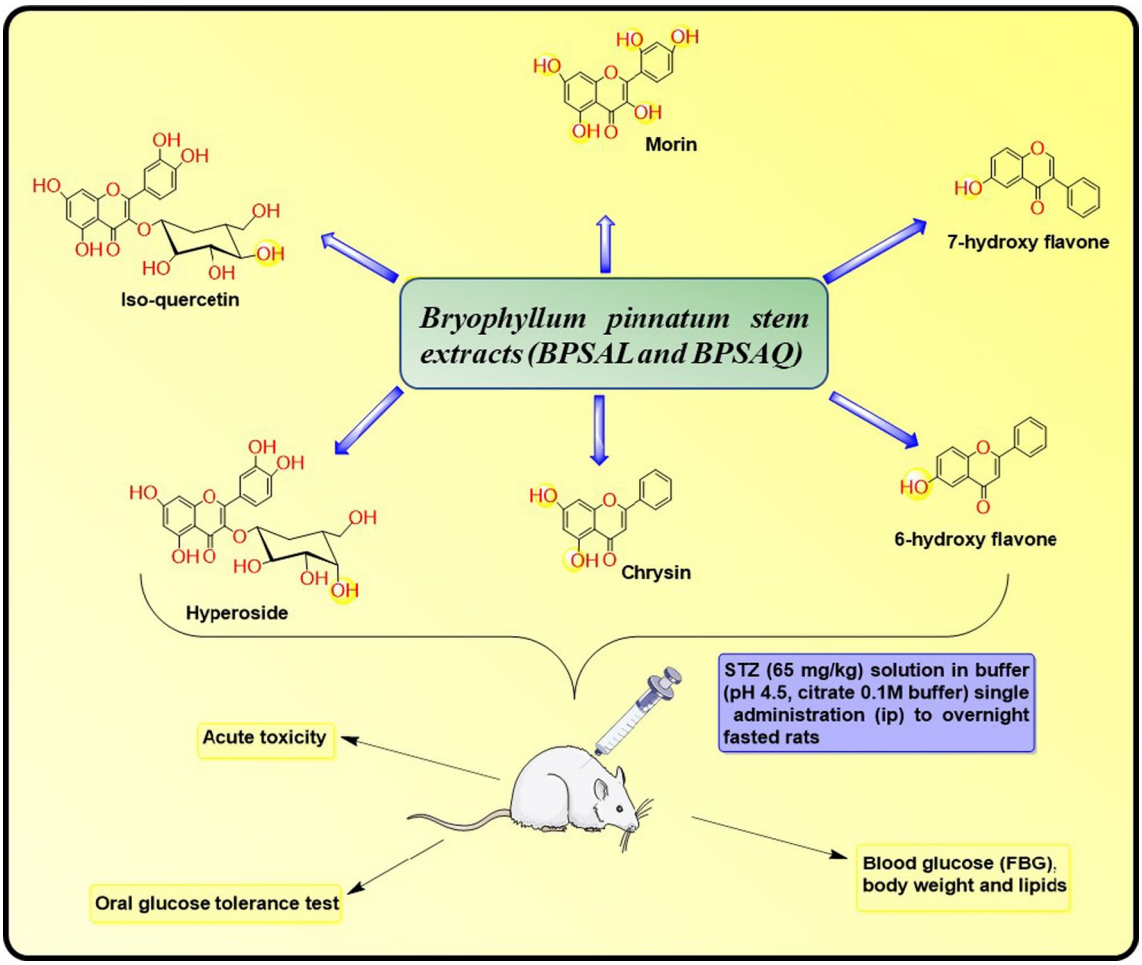
Graphical abstract



Background

Bryophyllum pinnatum, also known as Air Plant, Life Plant, Miracle Leaf, Goethe Plant, Katakataka, Zakhmehaiya, and Astibhaksha, is a miracle plant that belongs to the Crassulaceae family [1]. This plant can attain height of 1.5 m with hollow branching growth and can reach a height of 1–1.5 m and produces many bell-shaped blooms as shown in Fig. 1.

It is also known as the "wonder plant," because leaf itself can give rise to a new plant. This plant possesses number of therapeutic properties like insect bites, ulcers, wounds, inflammations and discolorations, rheumatic infections and boils, cancer, viral, smallpox, diarrhea, dysentery, lithiasis and phthisis, conjunctivitis, earache, and sore throats are all treated with this plant (Fig. 2) [2, 3].

This plant has been widely studied for its phytoconstituents due to its immense therapeutic qualities. The primary chemical constituents discovered in *B. pinnatum* are 3,4-dimethoxy quercetin (**1**) (Darmawan et al. 2013), 4′,5-dihydroxy-3′,8-dimethoxyflavone 7-*O*-D-glucopyradinoside, and 4′,5-dihydroxy-3′,8-dimethoxyflavone 7-*O*-D-glucopyradinoside (**2**), bryophollenone (**3**), bersaldegenin (**4**), bryophynol (**5**), bryophollone (**6**) [4], Bryophyllol (7,8), bryophyllin (**9,12**), etc. (Fig. 3) [5, 6].

The plant has also been reported as a rich source of minerals, particularly Zn, vital for maintaining one's health. Plant leaves and roots are reported to possess antidiabetic potential [7]. However, the stem extracts, have not yet been explored for any antidiabetic potential in STZ-induced type 2 diabetic rats. As a consequence, research into the antidiabetic potential of *B. pinnatum* stem extracts along with identification of

Fig. 1 *B. pinnatum* plant, twig, leaf and blooms

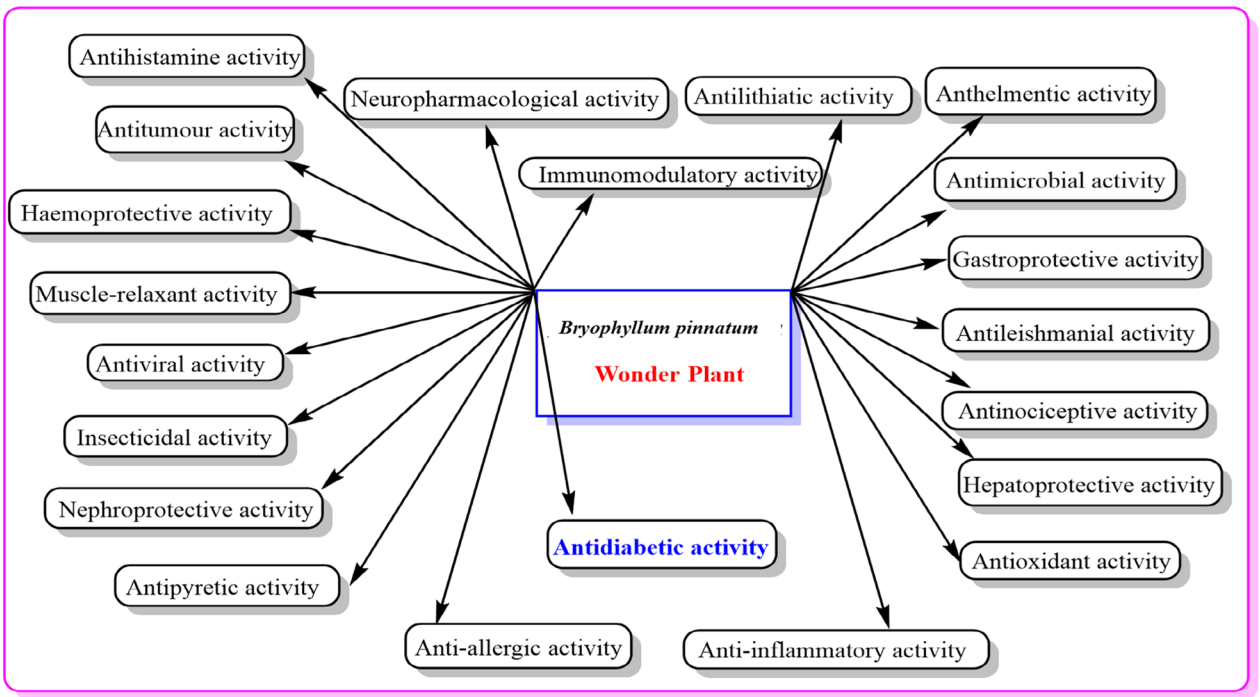


Fig. 2 Pharmacological activities of *B. pinnatum*, also known as "wonder plant"

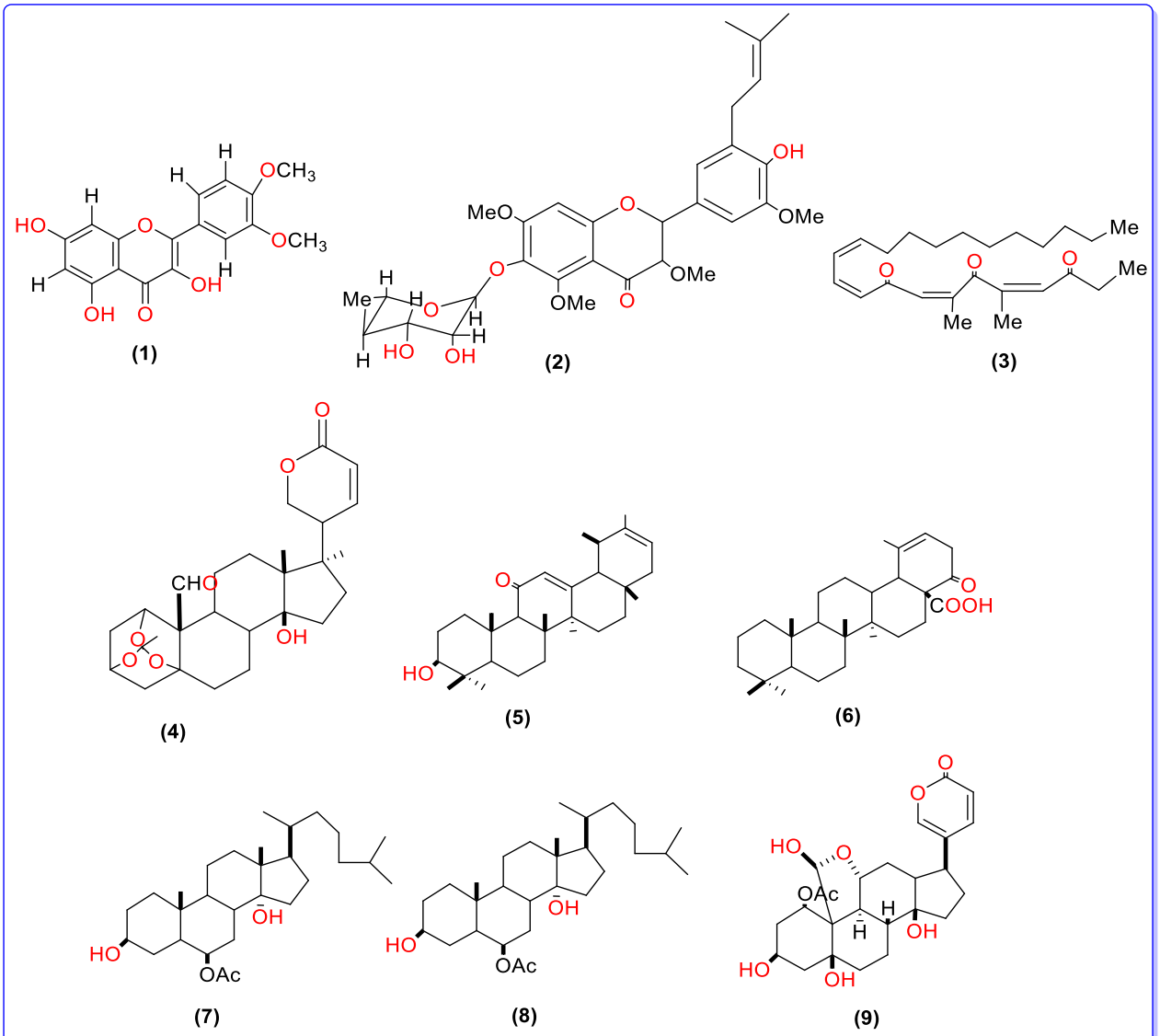


Fig. 3 Phyto-constituents of *B. pinnatum*. (1) 3-,4 4-dDimethoxy Quercetin (2) 4′,5-dihydroxy-3′,8-dimethoxyflavone 7-OD-glucopyradinoside (3) bryophollenone (4) bryophynol (5) bryophollone (6)) 5′,6-dihydroxy-3′,8-dimethoxyflavone 7-OD-glucopyradinoside (7) Bryophyllol (8) bryophyllin (9) bryophollone

its active phytoconstituents has been performed in the present work.

Methods

Chemicals

Sigma-Aldrich Co. (St. Louis, USA) provided the streptozotocin (STZ). Chemsynth laboratories Pvt. Ltd. (Haldwani, Uttarakhand, India) provided the solvents. $ZnSO_4$ has been purchased from Eisen-Golden brand in powder form of reagent grade. Span Diagnostic Ltd in India provided the analytical grade chemicals and biochemical kits.

Plant material

Stems of *B. pinnatum* were obtained from the herbal garden of Devsthali Vidyapeeth College of Pharmacy Rudrapur (Uttarakhand, India) in September. NBRI (Lucknow) has identified and authenticated the plant 2021. Herbarium specimen no. Varsha 030 was submitted at the Institute Museum.

Preparation of extracts

Fresh stems were cleaned thrice with water and dried at temperatures below 45 °C in a hot air oven. The BPSAL was prepared in a Soxhlet apparatus with 70% (v/v) alcohol for 48 h of continuous soxhlation followed by filtration and concentration. The mark has been dried before being transferred to a conical flask. BPSAQ has been prepared by using distilled water/$CHCl_3$ (9:1) by cold maceration (Fig. 4) [8].

Microscopic studies

Transverse sections of the stem were used to identify the characteristics of the plant and the stem [9].

Physicochemical investigations

Physiochemical analysis has been performed as per reference [8].

Preliminary phytochemical analysis (PPA)

Both extracts were submitted to PPA to identify their major phytoconstituents, and then a series of TLC assays have been undertaken to identify the best suitable mobile phase for further chromatographic analysis [9, 10].

Chromatographic examinations

Thin layer chromatography has been performed by using four different mobile phases in order to identify the major phytoconstituents:

Mobile Phase I: Ethyl acetate: Formic acid: Glacial acetic acid: water [10:1.1:1.1:2.6].

Mobile Phase II: Ethyl acetate: Methanol: Ethanol: Water [8.1:1.1: 0.4:0].

Mobile Phase III: Ethyl acetate: Butanone: Formic acid: Water [5:3:1:1].

Mobile Phase IV: Iso-butanol: Acetic acid: Water [14:1:3.5].

BPSAL, BPSAQ have been then subjected to fingerprinting analysis in order to identify their key phytoconstituents [11].

Pharmacological activities

Animals used

Male wistar rats weighing 150–180 g were procured from the IVRI in Bareilly (Uttar Pradesh, India). The animals were raised under ideal conditions of temperature (25 °C), humidity (55–65%), and a 12-h light–dark cycle. The animals had unlimited access to food and water. Prior to the commencement of the experiments, the animals were familiarized with the lab conditions. The

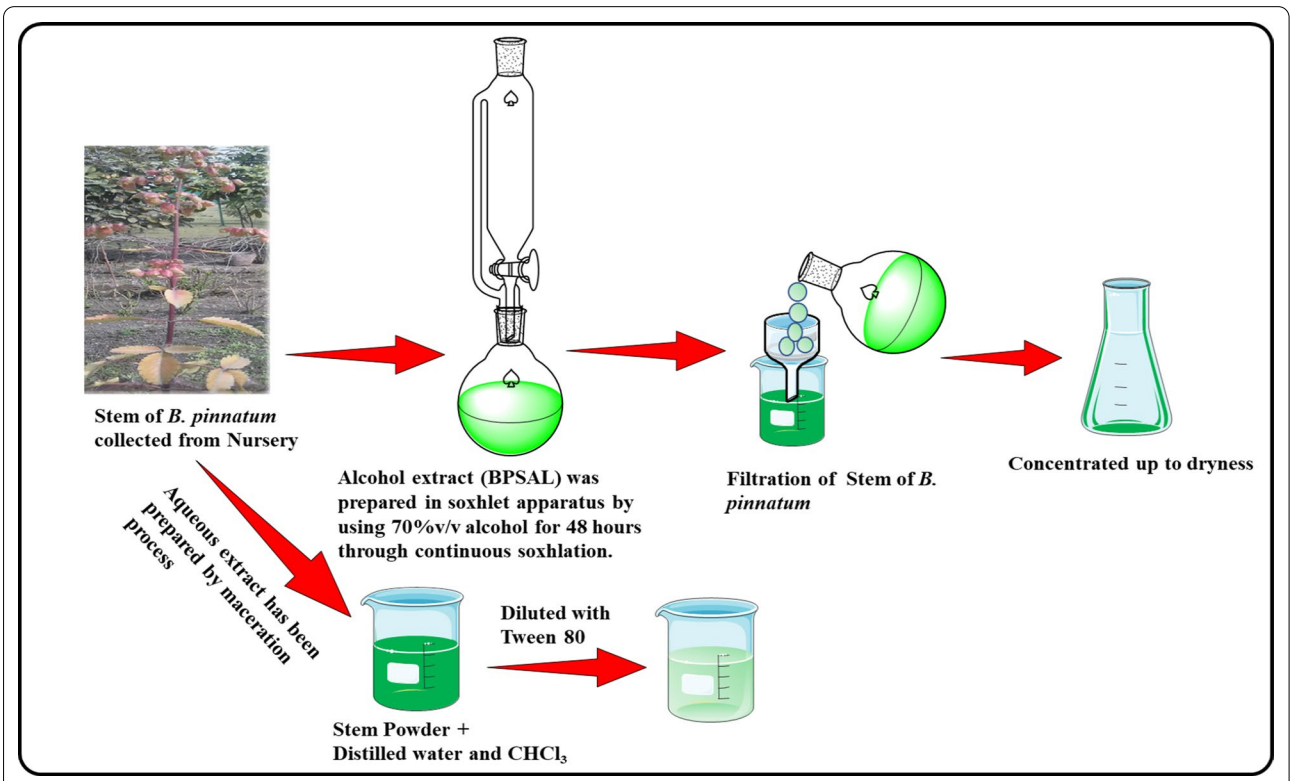


Fig. 4 Preparation of BPSAQ and BPSAL extracts

IAEC gave their approval to all of the tests. (CPCSEA/IAEC/2021-11/06).

Analysis of acute toxicity

The acute toxicity of the stem extracts has been performed as per OECD-420 guidelines. On healthyswiss albino mice (68 weeks) of either sex weighing 20–25 g were selected for toxicity analyses. Animal house was well maintained under standard hygienic conditions, at temperature (22 ± 2 °C), humidity (60% ± 10%) with 12 h day and night cycle, with food and water ad libitum. All pharmacological studies were carried out after obtaining approval from the Institutional Animal Ethics Committee of Devsthali Vidyapeeth College of Pharmacy (Rudrapur, Uttarakhand, India). The BPSAL, BPSAQ extracts were administered orally at doses of 5, 50, 300, 2000, 3000 mg/kg b.w. to the overnight fasted albino mice. Five animals were used for each dose and have been observed closely for the first 3 h, for any toxic manifestation (for example, increased motor activity, sedation, acute convulsion, coma and death). Thereafter, the observations were made at regular intervals for 24 h. The animals were under observation for 14 days [12, 13].

Anti-diabetic assessment

(a) Oral glucose tolerance test (**OGTT)**

Wistar rats have been divided into six groups, each with six animals, after being fasted overnight.

Group 1 was given 5% tween 80,

Group 2 was given Glibenclamide (10 mg/kg),

Groups 3 and 4 were given ethanol extracts at 200, 400 mg/kg bw.

Groups 5 and 6 were given aqueous extracts at 200, 400 mg/kg bw.

Groups 3, 4, 5, and 6 were administered glucose solution (2000 mg/kg) orally 30 min after extract administration. Blood was collected from the retro orbital sinus puncture before and after administration of the extracts at regular intervals of 30 min at 0, 30, 60, 90, and 120 min after glucose administration. Fasting blood glucose (FBG) has been determined using a glucose detection kit (Span diagnostic pvt. Ltd, Surat, India). [13–15].

(b)Induction of diabetes

A single i.p injection of a freshly prepared STZ (65 mg/kg) solution in buffer was used to induce diabetes (pH 4.5, citrate 0.1 M buffer). Blood samples were obtained from the retro orbital plexus 48 h after STZ injection, and plasma was separated from blood by centrifugation. FBG were tested using a GOD/POD kit to confirm diabetes.

Animals with diabetes were divided into 9 groups with six animals each.

Group 1: was provided with water and had unrestricted access to food and drink.

Group 2: The diabetic control group got 0.5 mL of 5% tween 80.

Group 3: Glibenclamide (10 mg/kg b.w.)

Groups 4,5: BPSAQ (200, 400 mg/kg b.w.)

Groups 6,7: BPSAL (200, 400 mg/kg b.w.)

Groups 8,9: BPSAL (200, 400 mg/kg b.w.) + $ZnSO_4$ (10 mg/dl).

The experiment has been performed up to 21 days. Blood samples were taken from overnight-fasting rats (retro-orbital sinus) at 0, 7, 14, and 21 days of the experiment before drug administration. On day 0 and day 21, the body weight of the rats was measured. On day 0 and day 21, biochemical tests were performed to evaluate the plasma lipid profile (Fig. 5) [13].

Statistical analysis

The data are presented in the form of a mean with standard deviation. For statistical analysis, two-way ANOVA and DMRT (Duncan's multiple range tests) were employed. The findings were deemed statistically significant when the value was $P < 0.05$ or below.

Fingerprinting analysis

Chromatographic analysis

Win-CATS4 software was used to run a Camag HPTLC system, Linomat V applicator, TLC scanner 3, Reprostar 3, and a 12-bit CCD camera for all of the images. Solvents for HPTLC were acquired from MERCK. All weighing was done with a computerized balance (Precisa XB 12A).

Sample preparation

Samples were prepared by dissolving the extracts in appropriate solutions subjected to sonication and centrifugation. A sample of the supernatant solution has been taken for analysis.

The samples were prepared using the above-mentioned method. For 30 min, the pre-coated plates were activated in a hot air oven at 120 °C. Spots of BPSAL and BPSAQ (2 µl) each have been placed on pre-coated (silica gel-60 F_{254}) plates with Linomat V applicator and Hamilton syringe triplicate (tracks 16). The chamber was saturated for at least 18 h, pre-coated plates with spots of BPSAL and BPSAQ were placed in chamber and allow to run up to a distance of 77 mm. The band length (8 mm), slit size, and scanning speed at 254 nm and 366 nm were scanned after the pre-coated plates were dried. Deuterium, tungsten, and mercury radiation sources were used for scanning of pre-coated plates at wavelengths of 254 nm, 366 nm, and 425 nm. Tables 1, 2 include fingerprinting

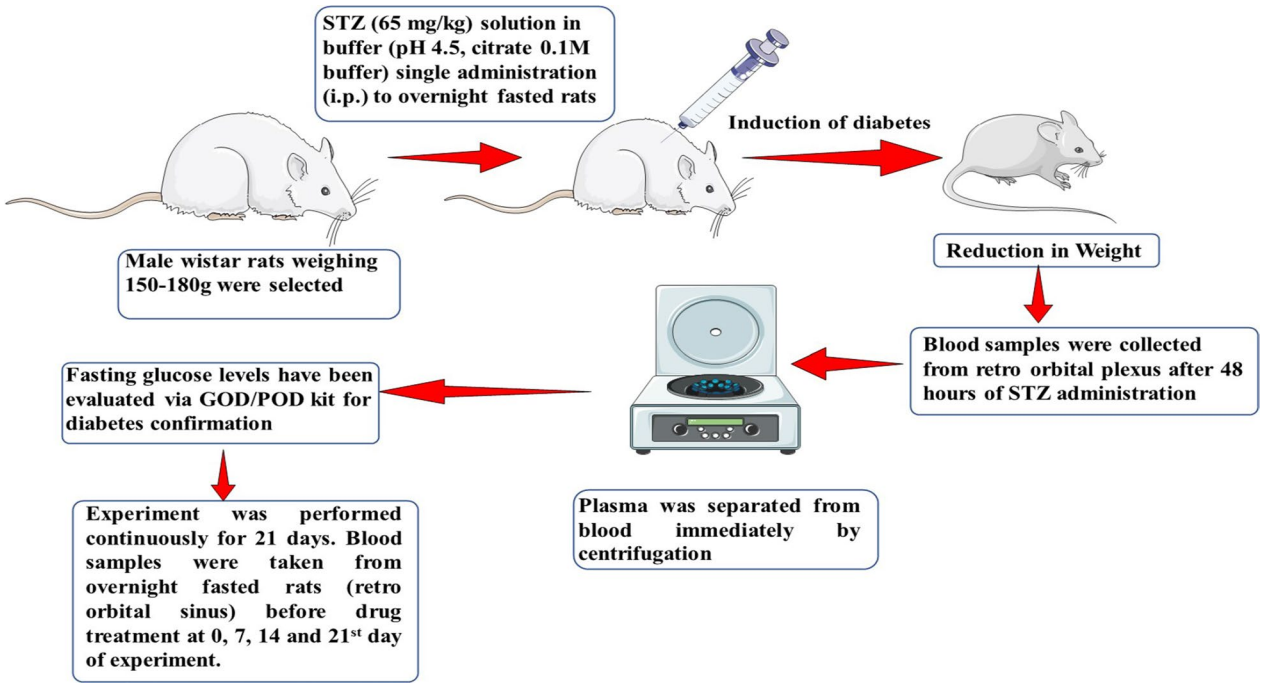


Fig. 5 Procedure of antidiabetic activity

Table 1 Parameters for fingerprinting analysis

Parameters	Values
Calibration parameters	
Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak height and area
Linomat 5 application parameters	
Spray gas	Inert gas
Sample solvent type	Methanol
Dosage speed	150 nl/s
Pre-dosage volume	0.2 µl
Syringe size	100 µl
Application position	8.0 mm
Band length	6.0 mm
Solvent front position	85.0 mm
Detection- camag TLC SCANNER	
Position of Track	10.0 mm
Number of Track	2
Distance between tracks	10.0 mm
Scan start position Y	5.0 mm
Scan end position Y	75.0 mm
Slit dimensions	4X0.3 mm, Micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step
Integration properties	
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest slope
Peak threshold min. slope	5
Peak threshold min. height	10AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	75.0 mm
Display scaling	Automatic
Measurement	
Wavelength	254 nm
Lamp	D_2& *W*
Measurement type	Remission
Measurement mode	Absorption
Optical filter	Second order
Detection mode	Automatic
PM high voltage	294 V

Table 2 Tracks representing sample position and volume

Track number	Application position (mm)	Application volume (µl)	Vial	Active
1	10.00	2.0	1	Yes
2	20.00	2.0	2	Yes

analysis specifications. A software was used to calculate the retention factor (Rf) and peak area. Table 1 shows the parameters used in fingerprinting analysis and Table 2 shows different tracks representing sample position and volume [7, 14, 16].

Results

Pharmacognostical studies

Macroscopical studies

The macroscopical characteristics of the plant sample received from a nursery were investigated. The stem displayed dark green hue, a distinct odor, a slightly bitter-pungent taste, and varied in size. On the outside, the stem has a thick cuticle and longitudinal lines/scars.

Microscopical studies

(a) Transverse section Uniseriate trichomes with thick epidermis were visible in a transverse section. A ring of vascular bundle patches, primary xylem, phloem, and very little pith area have been found just beneath the large cortical region. Small circular starch grains in a chain, representing slight secondary growth were observable. As shown in Fig. 6, the presence of starch granules in a specific characteristic pattern in the cortical area and a distinct pattern of phloem at the border of the vascular bundle ring were diagnostic characteristics of the plant.

Table 3 Treatment of stem sections with different reagents

S.N	Drug	Test for	Reagent	Reaction	Result
1	Section	Lignin	Phloroglucinol + dil HCl	Red color	+
2	Section	Mucilage	Ruthenium red solution	Red color	+
3	Section	Starch	Iodine solution	Blue color	+
4	Section	Fixed oil	Sudan red III solution	Red color	−
6	Section	crystals	Con. HCl	Effervescence	+

+ = present, − = absent

(b) Histochemical tests Sections when treated with different reagents like phloroglucinol, ruthenium red, iodine, sudan red III and conc. HCl showed the presence of lignin, mucilage, starch, fixed oil and crystals (Table 3).

Physicochemical constants

Parameters such as LOD, moisture content, Total ash, acid insoluble, water-soluble ash value, and extractive values (alcohol and water) of powdered drug were found to be 0.79, 12.5%, 9.58%, 2.45%, 5.68%, 8.94 and 12.64, respectively, as shown in Tables 4, 5 and 6.

Phytochemical analysis

Phytochemical analysis of Pet. Ether (60–80 °C), benzene, chloroform, acetone, alcohol and aqueous extracts has been performed. Phytosterols, fixed oils and fats were found to be present all extracts except acetone extracts. Alcohol extract was found to be rich in carbohydrates and glycosides, alkaloids, phenolic compounds and tannins, saponins and flavonoids. Aqueous extract was found to be rich in glycosides, phenolic compounds, flavonoids and saponins as shown in Table 7.

Fingerprinting analysis

Fingerprinting analysis of BPSAL extracts (2 µl) revealed 11 major peaks (R_f 0.07, 0.14, 0.15, 0.20, 0.25, 0.28, 0.44, 0.51, 0.63, 0.75, 0.81) when run in mobile Phase I; five sharp peaks (R_f 0.47, 0.60, 0.75, 0.77, 0.82) when run in mobile phase II (Cardiac glycosides); Six major peaks (R_f 0.02,0.05,0.14,0.35,0.36,0.61) in mobile phase III; five prominent peaks (R_f 0.06, 0.16, 0.28, 0.42, 0.72) in mobile phase IV in Tables 8, 9, 10 and 11, Figs. 7 and 8A,C.

Among the above major peaks, peak at R_f 0.42 was identified to 7-hydroxy flavone, peaks at R_f at 0.14, 0.61 were identified as morin and hydroxy flavone, respectively, when compared with standard R_f [17]. Spots at R_f 0.25 and 0.81 were identified as terpenoids (I & II), respectively, in BPSAL [18].

Fingerprinting analysis of BPSAQ (2 µl) revealed 8 prominent peaks (R_f 0.07, 0.11, 0.28, 0.44, 0.57, 0.62, 0.64, 0.71) when run in mobile phase I; 2 prominent peaks (R_f 0.01, 0.77) when run in mobile phase II; 4 major peaks (R_f 0.01, 0.09, 0.60,0.68) when run in mobile phase III; 3 sharp peaks at R_f 0.06, 0.17, 0.73 in mobile phase IV, respectively, as shown in Tables 12, 13, 14 and 15, Fig. 7 and 8B, D. Among above-mentioned R_f, peak at R_f 0.60 shows presence of Chrysin in aqueous extract when compared with standard R_f [17]. Spots corresponding to R_f 0.77 and 0.68 showed the presence of Iso-quercetin and hyperosides, respectively, in aqueous extract when compared with standard R_f [19].

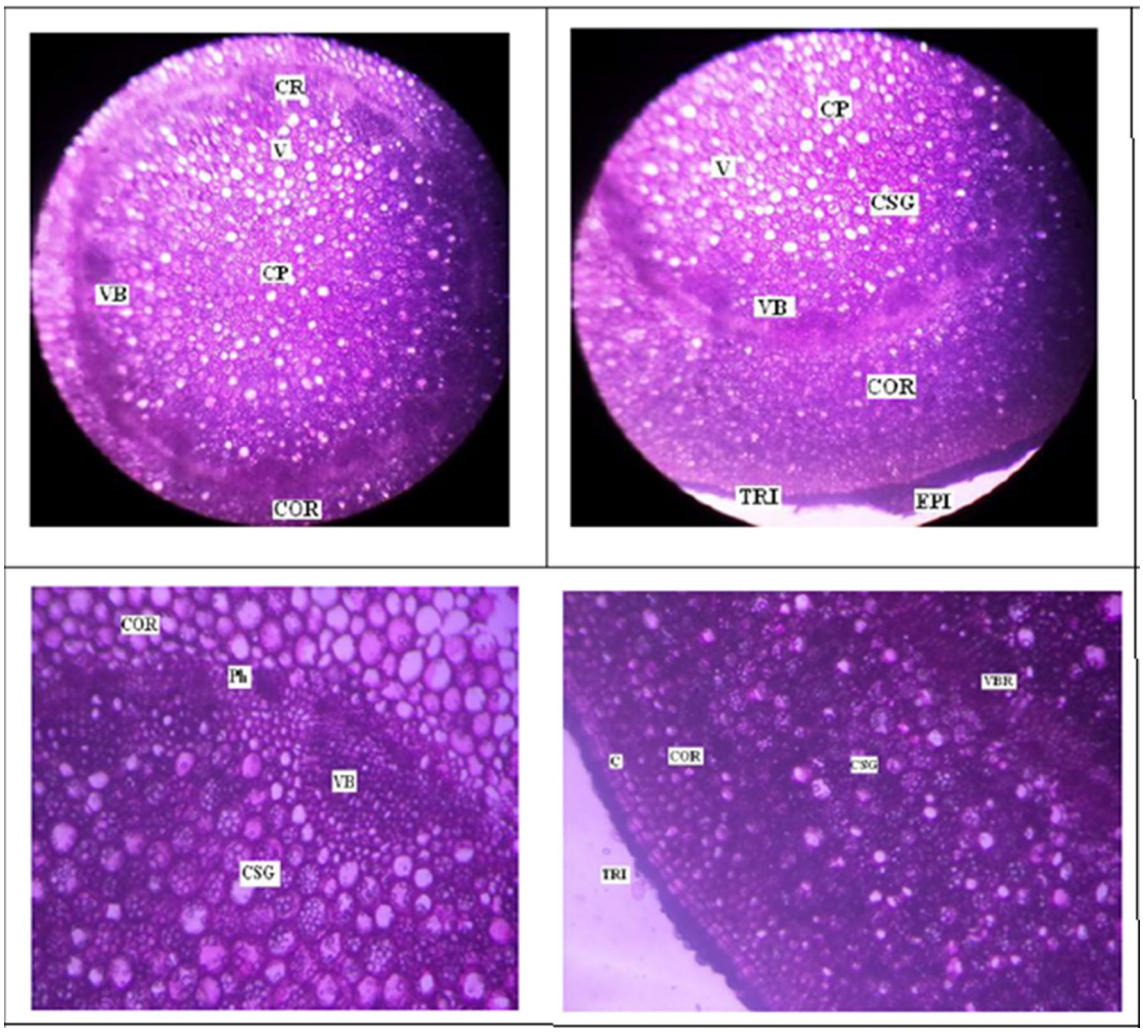


Fig. 6 The transverse sections of stem of *B. pinnatum*. T.s of plant showed the presence of vascular bundles, cortex, trichomes, starch grains as diagnostic charcateristics

Table 4 Moisture content of *B. pinnatum* stem

Fresh weight (g)	Dry weight (g)	Loss on drying (g)	Moisture content (%)
2.00	1.21	0.79	12.5

Table 5 Ash values of *B. pinnatum* stem

Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)
9.58	2.45	5.68

Fingerprinting analysis results showed that aqueous extract is rich in flavonoids and phenol constituents. BPSAQ showed major spots at R_f 0.09, 0.17, 0.23, 0.60, 0.68, 0.73, 0.77, 0.86 quenched fluorescence at 254 nm and 366 nm, respectively. All spots quenched fluorescence at 254 nm and showed blue and violet fluorescence at 366 nm. Figure 9 shows the three-dimensional view of extracts in all the four mobile phases. BPSAL showed 6 peaks, whereas BPSAQ showed 4 peaks. Peaks with R_f 0.14 and 0.61 in BPSAL and 0.60 in BPSAQ corresponds to morin, 6-hydroxy flavone and chrysin, respectively [20].

Table 6 Extractive values of *B. pinnatum* stem

S. NO	Extractives	Extractive values (% w/w)
1	Alcohol soluble	8.94
2	Water soluble	12.64

Table 7 Preliminary phytochemical analysis of *B. pinnatum* stem

S.N	Test for	Pet. Ether extract	Benzene extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
1	Alkaloids	−	−	−	−	+	+
2	Carbohydrates and glycosides	−	−	+	−	+	+
3	Phyto-sterols	+	+	+	+	+	+
4	Fixed oils and Fats	+	+	+	−	+	+
5	Phenolic compounds and tannins	−	−	−	+	+	+
6	Saponins	−	−	−	−	−	+
7	Flavonoids	−	−	−	−	+	+
8	Gums and mucilage	−	−	−	−	−	+
9	Volatile oils	−	−	−	−	−	−

+ = present, − = absent

Table 8 Peak table of BPSAL in Mobile Phase I

Peak	R_f	Height	Area	Assigned substance
1	0.07	39.2	609.9	Unknown
2	0.14	18.6	218.4	Unknown
3	0.15	15.5	168.8	Unknown
4	0.20	12.1	120.6	Unknown
5	0.25	13.7	229.6	Terpenoid 1**
6	0.28	18.2	352.9	Unknown
7	0.44	28.4	396.5	Unknown
8	0.51	81.1	3399.9	Unknown
9	0.63	21.3	790.7	Unknown
10	0.75	150.6	9772.7	Unknown
11	0.81	140.0	5388.4	Terpenoid 2**

**When compared in similar conditions[21]

Table 9 Peak table of BPSAL in mobile phase-II

Peak	R_f	Height	Area	Assigned substance
1	0.47	24.6	198.0	Unknown
2	0.60	21.1	904.1	Unknown
3	0.75	65.3	2242.6	Unknown
4	0.77	39.0	312.7	Unknown
5	0.82	48.7	524.1	Unknown

Table 10 Peak table of BPSAL in mobile phase III

Peak	R_f	Height	Area	Assigned substance
1	0.02	767.9	10,143.5	Unknown
2	0.05	102.8	2377.1	Unknown
3	0.14	13.7	329.4	Morin* (10)
4	0.35	16.8	612.9	Unknown
5	0.36	17.7	255	Unknown
6	0.61	17.3	490.2	6-hydroxy flavone** (11)

*,**When compared in similar conditions[22, 23]

Table 11 Peak table of BPSAL in mobile phase IV

Peak	R_f	Height	Area	Assigned substance
1	0.06	66.1	2170	Unknown
2	0.16	62.3	2349	Unknown
3	0.28	103.1	8006.0	Unknown
4	0.42	142.7	5927	7-hydroxy flavone* (12)
5	0.72	681.7	55,533.0	Unknown

*When compared in similar conditions [22, 23]

Pharmacological activity

Acute toxicity studies

Toxicity analysis of extracts has been found to be safe up to dose of 3000 mg/kg b.w.

OGTT of BPSAL and BPSAQ extracts

Oral glucose tolerance analysis of BPSAL and BPSAQ is shown in Fig. 10. BPSAL and BPSAQ (200 and 400 mg/kg b. w) have not significantly reduced the FBG in OGTT analysis. This means the extracts do not exert significant effects on insulin secretion.

Anti-diabetic activity

Anti-diabetic analysis results of BPSAL, BPSAQ are shown in Fig. 11. Both the extracts at 200, 400 mg/kg b.w. have significantly reduced the elevated FBG in diabetic rats. When compared among the extracts, BPSAQ at 400 mg/kg b.w. has shown maximum antidiabetic activity. BPSAL (200, 400 mg/kg b.w.) revealed the statistically significant activity ($p > 0.05$) when combined with $ZnSO_4$ synergistically showed the marked decrease in FBG ($p > 0.001$) in STZ-induced diabetic studies. $ZnSO_4$ synergistically augmented the antidiabetic potential of BPSAL comparable with that of standard drug. BPSAL + $ZnSO_4$ (200,400 mg/kgbw) showed statistically significant

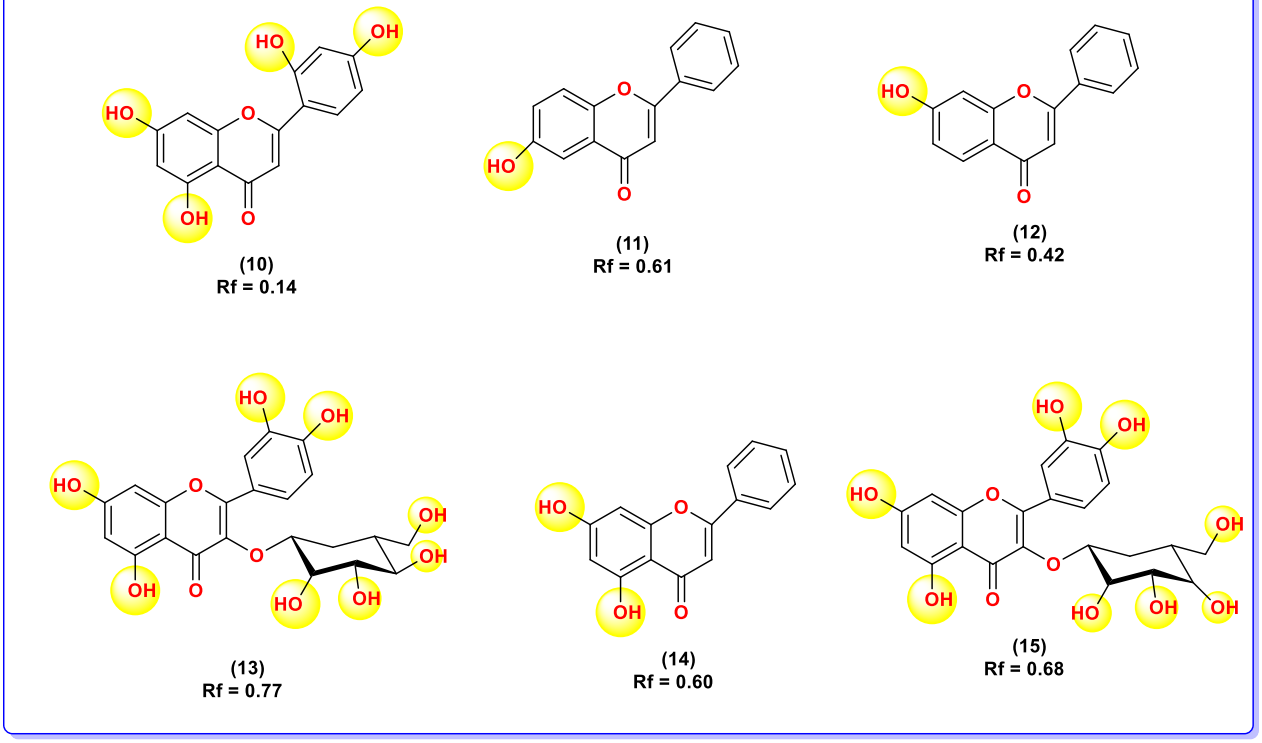


Fig. 7 Identified Phyto-constituents of *B. pinnatum*. R_f 0.60 Chrysin, R_f 0.77, 0.68 Iso-quercetin and hyperosides, R_f 0.42 7-hydroxy flavone, R_f at 0.14, 0.61 morin and 6-hydroxy flavone

reduction in LDL, TG and TC levels and elevated level of HDL when compared among all extracts (Fig. 12). BPASAL and BPSAQ does not show any weight gain, but $ZnSO_4$ also showed synergistic effect on weight (Fig. 13).

Discussion

Macroscopical studies showed dark green stem, distinct odor, a slightly bitter-pungent taste, and varied in size. On the outside, the stem has a thick cuticle and longitudinal lines/scars. Microscopical studies showed presence pf the thick cuticle and longitudinal lines/scars, Uniseriate trichomes with thick epidermis, ring of vascular bundle patches, primary xylem, phloem, and very little pith area have been found just beneath the large cortical region. Physiochemical analysis standardization of plant will be helpful in correct identification of plant in powdered form. Ash value, extractive value, histochemical analysis and microscopical analysis will be helpful in identification of correct species as well as variety.

Phytochemical analysis of Pet. Ether (60–80 °C), benzene, chloroform, acetone, alcohol and aqueous extracts have been performed. Phytosterols, fixed oils and fats were found to be present all extracts except acetone extracts. Alcohol extract was found to be rich in carbohydrates and glycosides, alkaloids, phenolic compounds and tannins, saponins and flavonoids. Aqueous extract was found to be rich in glycosides, phenolic compounds, flavonoids and saponins. Major phytoconstituents detected in fingerprinting analysis are morin, chrysin, 6-hydroxy flavones, iso-quercetin and hyperosides from flavonoid category and terpenoids. These flavonoids are reported to possess significant antidiabetic potential. Morin improves fructose-induced hepatic SphK1/S1P signaling pathway impairment, leads to reduction of hepatic NF-κB activation with IL-1b, IL-6 and TNF levels. Moreover, morin also reduces the lipid accumulation and reduces hyperlipidemia and recovered insulin and leptin sensitivity (Fig. 14).

Quercetin has been reported to act through many cellular mechanisms increasing SOD, GPX, CAT and inhibition of PI3K and GLUT2 receptors, blockade of tyrosine kinase, stimulation of GLUT4 in skeletal muscles and also act through AMPK activation. Isoflavones and phenolic phytoconstituents also play significant role to maintain glucose homeostasis through inhibition of tyrosine kinase enzyme and promoting cAMP/PKA signaling pathway [21].

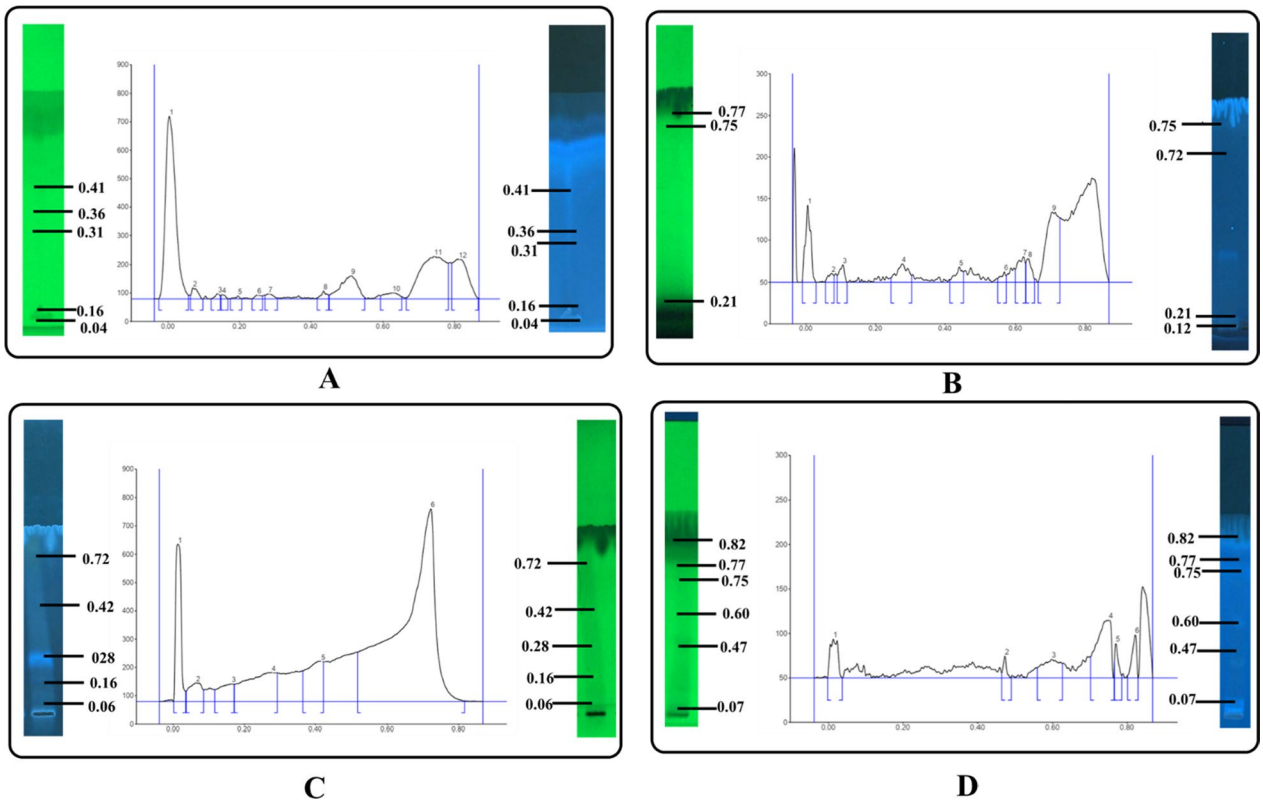


Fig. 8 HPTLC fingerprint chromatogram of BPSAL in Mobile Phase **A** $CHCl_3$:C_2H_5OH [9.8:0.2]) **C** Toluene: Ethyl acetate: Glacial acetic acid [5:7:1]) and BPSAQ in Mobile Phase (**B**) Toluene: Ethyl acetate: Glacial acetic acid [5:7:1], **D** $CHCl_3$: CH_3OH [95:5])

Table 12 Peak table of BPSAQ in mobile phase I

Peak	R_f	Height	Area	Assigned substance
1	0.07	10.7	156.4	Unknown
2	0.11	21.4	280.7	Unknown
3	0.28	22.3	650.9	Unknown
4	0.44	18.6	327.3	Unknown
5	0.57	12.0	161.2	Unknown
6	0.62	30.9	591.5	Unknown
7	0.64	28.5	382.2	Unknown
8	0.71	84.1	2767.8	Unknown

Table 13 Peak table of BPSAQ in Mobile Phase II

Peak	R_f	Height	Area	Assigned substance
1	0.01	266.4	2815.3	Unknown
2	0.77	21.4	624.7	Iso-quercetin***

***When compared in similar conditions [13, 26]

Both the extracts were found to be safe up to the doses of 3000 mg/kg bw. Oral glucose tolerance analysis of BPSAL and BPSAQ (200 and 400 mg/kg b. w) have not

Table 14 Peak table of BPSAQ in mobile phase III

Peak	R_f	Height	Area	Assigned substance
1	0.01	68.4	1218.0	Unknown
2	0.09	10.5	270.0	Unknown
3	0.60	31.4	1038.0	Chrysin* (14)
4	0.68	22.7	572.3	Hyperoside***(15)

*When compared in similar conditions [22, 23], ***When compared in similar conditions [13, 26]

Table 15 Peak table of BPSAQ in mobile phase IV

Peak	R_f	Height	Area	Assigned substance
1	0.06	37.5	888.3	Unknown
2	0.17	59.3	2400.0	Unknown
3	0.73	682.8	64,506.6	Unknown

significantly reduced the FBG in OGTT analysis. This indicates that extracts do not exert significant effects on insulin secretion. Antidiabetic potential of BPSAL, BPSAL + Zn and BPSAQ has been evaluated by STZ-induced models. BPSAL and BPSAQ does not show

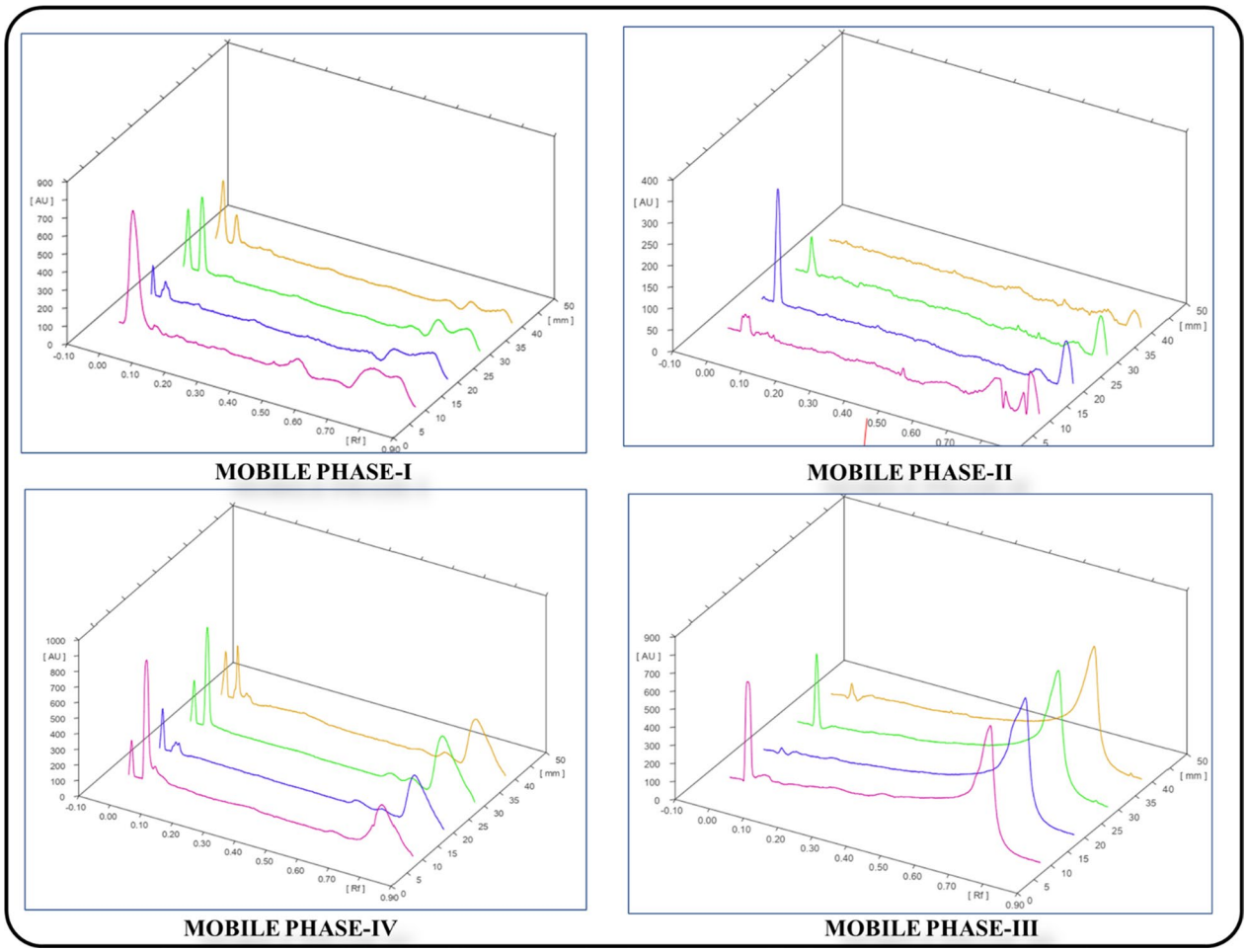


Fig. 9 Three-dimensional view of extracts in various mobile phases in four different mobile phases. Values are expressed as Mean ± S.E.M ($n = 6$); ** = $P < 0.001$ significant from diabetic control group

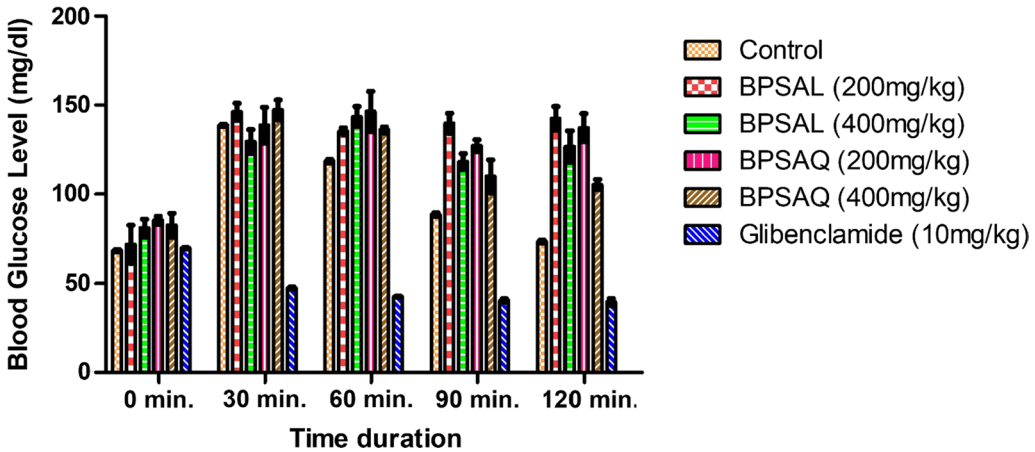


Fig. 10 OGTT results of BPSAL and BPSAQ on FBG. Values are expressed as Mean ± S.E.M ($n = 6$); ** = $P < 0.001$ significant from diabetic control group

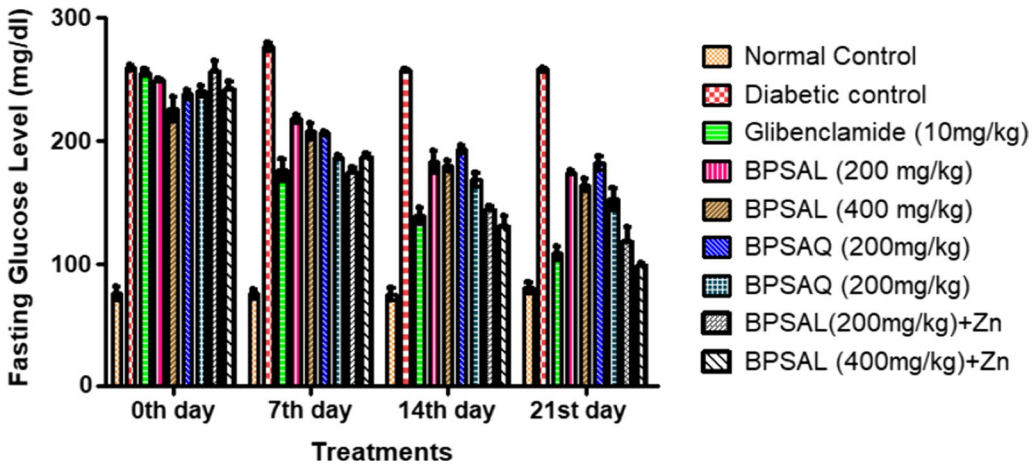


Fig. 11 Effect of extracts of *B. pinnatum* on FBG in streptozotocin-induced diabetic rats. Values are expressed as Mean ± S.E.M ($n = 6$); ** = $P < 0.001$ significant from diabetic control group

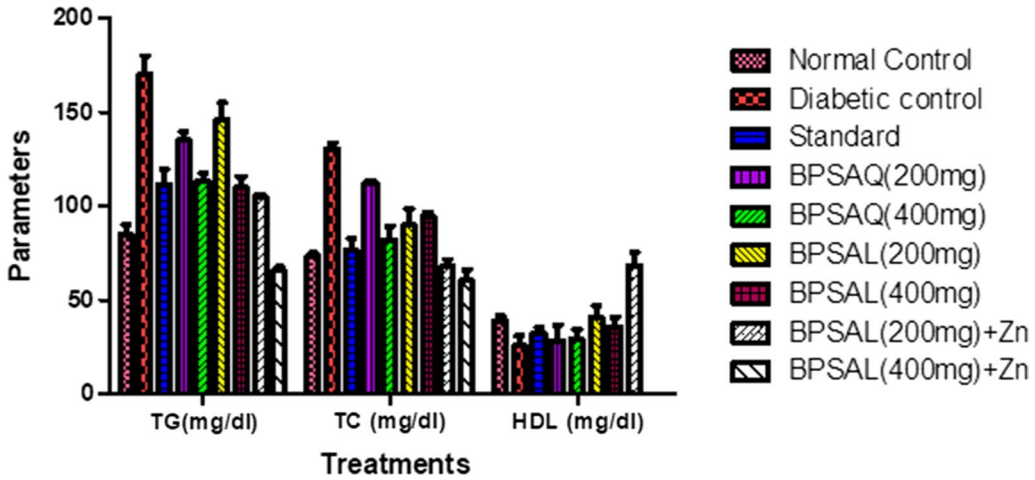


Fig. 12 Effect of extracts of *B. pinnatum* on lipid profile in streptozotocin-induced diabetic rats. Values are expressed as Mean ± S.E.M ($n = 6$); ** = $P < 0.001$ significant from diabetic control group

significant effect in reducing the FBG in OGTT analysis but showed significant reduction in FBG, LDL, TG and TC in STZ-induced diabetic analysis revealed its effects similar to Biguanides, which don't enhance the insulin secretion but facilitate glucose absorption, reduces the glucose release from liver, better be called as "antihyperglycemic" rather than "hypoglycemic".

The results indicates that extracts may acts as increasing the insulin sensitivity by increasing the glucose uptake through cells by various mechanisms similar to biguanides. Possible mechanism behind the observed significant antidiabetic potential lies in AMPK activation leads to suppression of anabolism and stimulation of catabolism leads to phosphorylation of cAMP specific 3′,5′-cyclic PDE4B and activates cAMP degradation. Consequently, it prevents the activation of cAMP-dependent PKA which further activates CREB-CBP-CRTC2 (CREB: CRTC2) transcription complex, PEPCK and G6Pase. AMPK also inhibits SREBP-1 which lead to reduction of FAS and SCD1 and ME and therefore inhibits fatty acid synthesis. Zn activates the AMPK through MKKβ, imparts synergistic role and further potentiates the antidiabetic potential of extracts [22, 23]. Figure 15.

Among all the extract BPSAL + $ZnSO_4$ revealed most significant effect and reduces the LDL, TG and TC level and comparable with that of standard drug. Zn is essential for adequate function of most of the

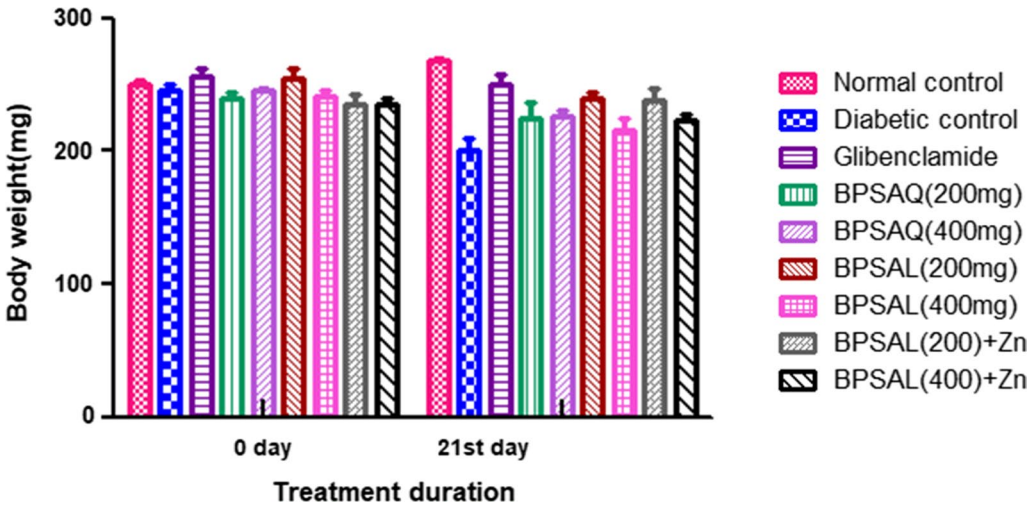


Fig. 13 Effect of *B. pinnatum* extracts on body weight of rats. Values are expressed as Mean± S.E.M ($n = 6$); ** = $P < 0.001$ significant from diabetic control group

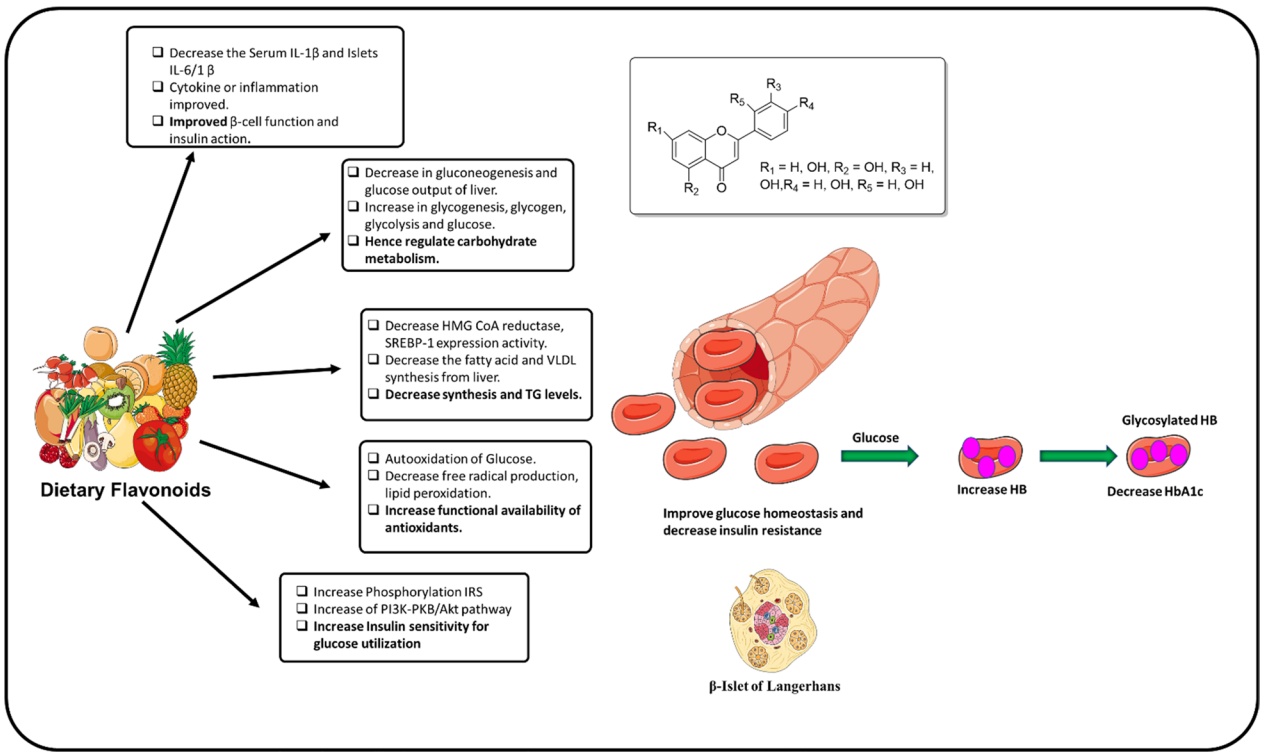


Fig. 14 Sources of dietary flavonoids. These flavonoids possess antidiabetic and antioxidant effects by various mechanisms such as inhibition of serum IL-1β and IL-6/1β, enhancing the β-islets cells, inhibition of gluconeogenesis and glucose release in blood from liver, stimulation of glycogenesis, inhibition of HMG-CoA reductase and SREBP-1, reduction in the level of triglycerides, stimulation of glucose sensitivity by stimulation of phosphorylation IRS and PI3K-PKB/Akt pathways, etc.

hormones, there are two prominent mechanism of action that may explain the significant antidiabetic action of BPSAL + $ZnSO_4$. Zn interacts with insulin in three ways, i.e., (a) it binds with insulin in pancreas and stops its entry in blood, whenever not required, (b) it enhances the glucose absorption through by

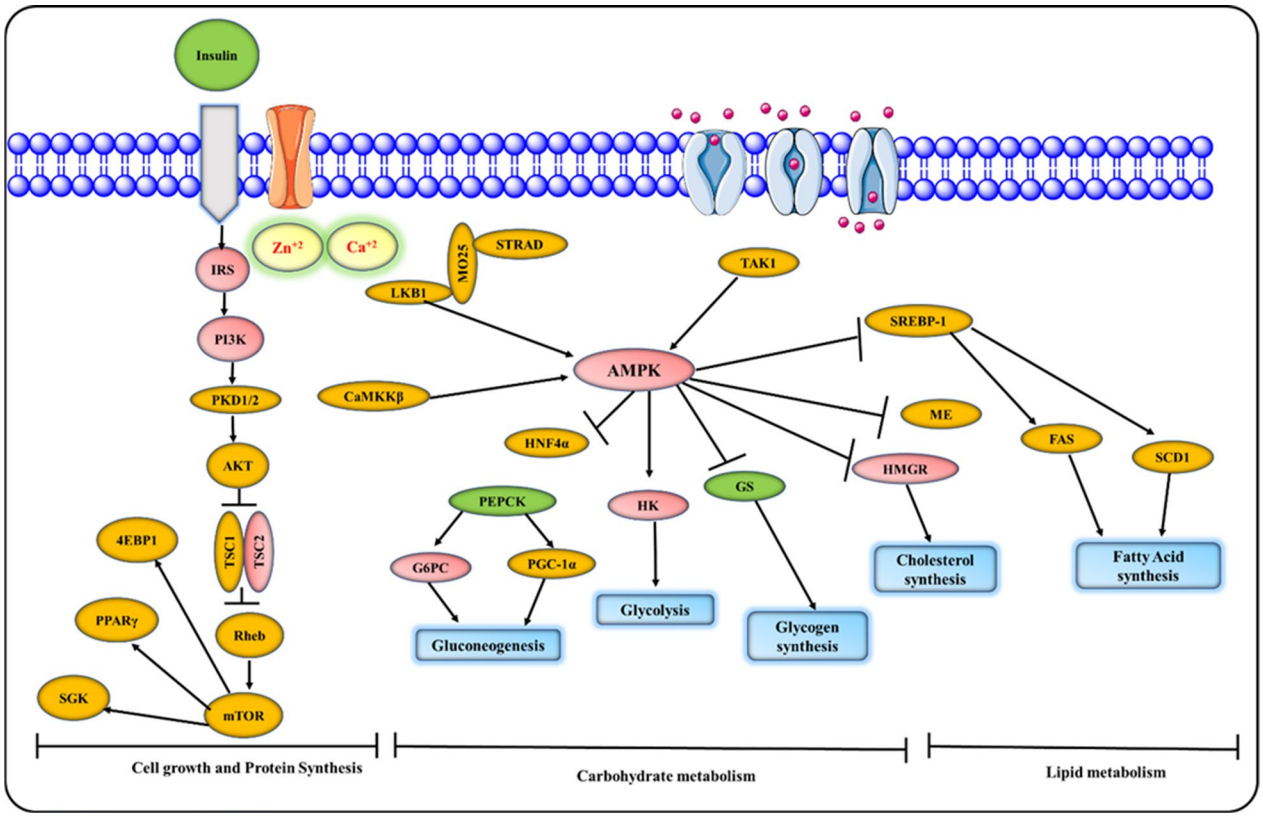


Fig. 15 Mechanism of action of extracts through AMPK activation results in increase in glucose uptake, inhibition of gluconeogenesis and fatty acid synthesis. Phosphoenolpyruvate carboxykinase = PEPCK, Protein kinase A = PKA, Glucose 6-phosphatase = G6Pase, Phosphodiesterase 4B = PDE4B, AMP-activated protein kinase = AMPK, Liver kinase B1 = LKB1, Mouse protein-25 = MO-25, Lep R = Leptin R, Protein Phosphatase 2A = PP 2A, Sterol regulatory element-binding proteins = SREBPs, Stearoyl-CoA Desaturase-1 = SCD1, HMG-CoA Reductase = 3-hydroxy-3-methyl-glutaryl-CoA reductase = HMGR, Carnitine Palmitoyl-transferase I = CPT1, Phosphofructokinase 2 = PFK2, Phosphoenolpyruvate carboxykinase = PEPCK, Mammalian target of rapamycin = mTOR

enhancing the binding of insulin to cells and fastens the glucose uptake inside the cells correctly known as "insulin sensitivity", and (c) zinc showed marked anti-inflammatory potential degrade inflammatory markers (C-reactive proteins reduces the edema in cells and retains cell health as well as insulin sensitivity).

STZ destroys β-pancreatic cells via alkylation of DNA, entering through $GLUT_2$ receptors, which in turn damages DNA through inducing ADP-ribosylation turns into NAD^+ depletion, ATP within the cell results in diabetes. Besides, it also generates H_2O_2, OH radicals and toxic chemicals like nitric oxide that results in DNA damage in β-pancreatic cells.

Role of oxidative stress cannot be denied in diabetes; reactive oxygen species such as OH^-, O_2^-, H_2O_2, NO^- produced during metabolism may degrade protein and enzymes, plasmids, inhibiting protein synthesis, plasma membrane disruption and DNA degradation. ROS may hamper mitochondrial functions and trigger apoptotic pathways and also facilitate cell lysis through necrosis and apoptosis. ROS excessive production leads to oxidative DNA damage/mitotic death may also trigger autophagic/mitophagic cell death. Zn is required for synthesis of superoxide dismutase enzymes which in turn reduces the oxidative stress (Fig. 15). Besides it, Zn has also been reported to possess numerous therapeutic potentials such as anti-inflammatory, photocatalytic, wound healing, anticancer, antioxidant, antifungal, antibacterial activities, etc. [24].

Flavonoids, polyphenols, glycosides, steroids, tri-terpenoids, and phytosterols were found in BPSAQ and BPSAL, suggesting that they may help with antidiabetic activity [25, 26]. The presence of Zn in the extract could explain the substantial antihyperglycemic effect. It has previously been noted that this plant contains Zn, and some studies have shown that Zn supplementation can help diabetic patients control their blood glucose levels [27, 28].

Significant weight loss is a hallmark of STZ-induced diabetes, which was also seen in the present study. The BPSAL (400 mg/kg) and glibenclamide treated groups exhibited a substantial improvement in body weight ($p < 0.05$) when compared to the normal control group, but other extracts did not fully restore the weight loss. BPSAL + $ZnSO_4$ showed statistically significant effects on body weight showed synergistic effect of Zn when combined with BPSAL.

The mechanism of action of Zn may involve binding with IGF-I, which facilitates diverse cell events like amino acid stimulation, glucose uptake, and cell cycle regulation; it also associates with a membrane-associated receptor with tyrosine kinase activity. When IGF-I activates the receptor, a sequence of phosphorylation takes place within the cell, mediating cellular functions. Insulin-like growth factor-I binds to IGF binding proteins in the circulation; at least eight distinct binding proteins have thus far been recognized. There is mounting evidence that IGF-I binding proteins are not inert carrier proteins, but rather part of a complicated system that regulates IGF-I availability to cells. Nutritional status has

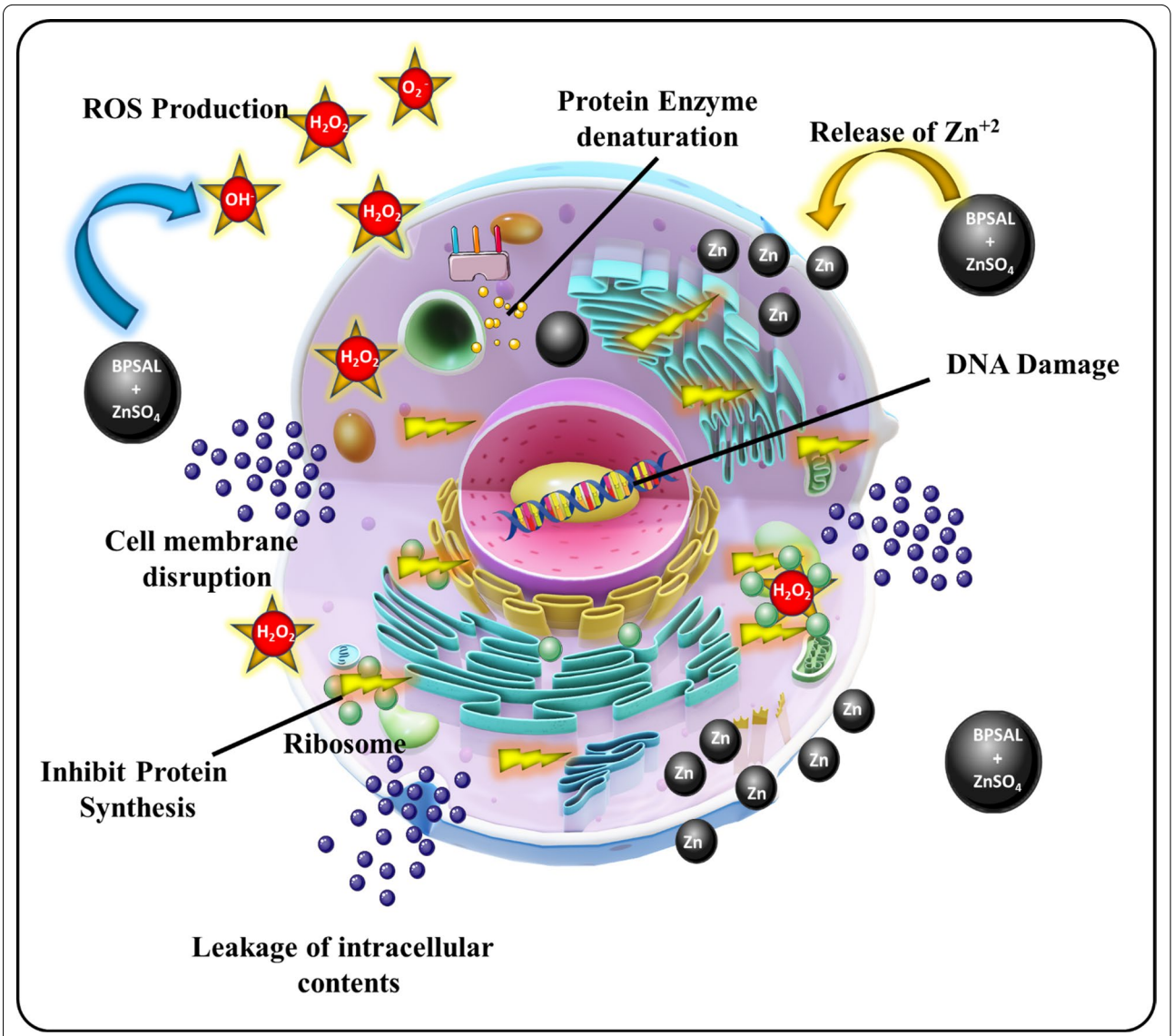


Fig. 16 Role of zinc ion in diabetes. BPSAL along with $ZnSO_4$ when enters into the cells converts into Zn + + ions and extracts when enters into the cell disturb the cell potential and positive ions inside the cell capture the various ROS such as OH^-, NO^-, H_2O_2, etc., which causes the degradation of ribosomes, cell membrane disruption, DNA damage and protein denaturation etc.

a strong influence on IGF-I levels in the blood. In particular, decreased serum IGF-I was observed in humans and animals in case of Zn deficiency (Fig. 16).

Therefore, $ZnSO_4$ showed synergistic effect on antidiabetic potential of BPSAL that may act through various mechanism. Further studies are required to elucidate more specific mechanism of action of antidiabetic potential of extract with $ZnSO_4$ [29].

Conclusion

The current study investigates the acute toxicity, antidiabetic, fingerprinting, and pharmacognostical evaluation of the *B. pinnatum* stem. Fingerprinting analysis reveals the presence of morin, chrysin, 6-hydroxy flavones, isoquercetin and hyperosides flavonoid category and terpenoids are reported to possess significant antioxidant and antidiabetic potential through various mechanism such as SphK1/S1P signaling pathway impairment, leads to reduction of hepatic NF-κB activation, TNF-α, increasing SOD, GPX, CAT and inhibition of PI3K and GLUT2 receptors, blockade of tyrosine kinase, stimulation of GLUT4 in skeletal muscles and also act through AMPK activation (Fig. 16). Flavanoids such as quercetin, isoflavones, etc., may act through AMPK activation and stimulation of catabolism leads to phosphorylation of cAMP specific 3′,5′-cyclic PDE4B and activates cAMP breakdown. Subsequently, hampers cAMP-dependent PKA activation leads to activation of CREB-CBP-CRTC2 (CREB: CRTC2) transcription complex, PEPCK and G6Pase. Activated AMPK inhibits TORC2 and HNF4α which leads to effect of G6PC and PEPCK results in gluconeogenesis stimulation and reduces the blood glucose level. Zn activates the AMPK through MKKβ imparting synergistic role and further potentiates the antidiabetic potential of extracts.

ROS such as OH^-, O_2^-, H_2O_2, NO^- generated in metabolism may degrade protein and enzymes, plasmids, inhibits protein synthesis, plasma membrane disruption and DNA degradation. Zn is required for synthesis of Superoxide dismutase enzymes in turn reduces the oxidative stress. The mechanism of action of Zn may also involve in binding with IGF-I, which facilitates diverse cell events like amino acid stimulation, glucose uptake, and cell cycle regulation; it also associates with a membrane-associated receptor with tyrosine kinase activity. In acute toxicity testing, both extracts (BPSAL and BPSAQ) were determined to be safe, even at a high dose of 3000 mg/kg b.w. Both the extracts did not show significant potential in OGTT analysis but showed significant antidiabetic potential in STZ-induced diabetic evaluation. The antidiabetic potential was further enhanced when combined with $ZnSO_4$.

The possible mechanism of action has been proposed but further studies are required to elaborate the exact mechanism of action at molecular level. The extract contains morin, chrysin and 6-hydroxy flavones, as well as iso-quercetin, terpenoids 1, 2, and hyperosides as confirmed in fingerprinting analysis. The antihyperglycemic action may be due to secondary metabolites in conjunction with minerals. More study is required to identify the precise mechanism of action and phytoconstituents responsible for the antihyperglycemic effect.

Abbreviations

B. pinnata: *Bryophyllum pinnata*; OGTT: Oral glucose tolerance test; BGL: Blood plasma glucose level; STZ: Streptozotocin; BPSAL: Alcohol extract; LOD: Loss on drying; BPSAQ: Aqueous extract; FBG: Fasting blood glucose; DM: Diabetes mellitus; TG: Triglycerides; b.w.: Body weight; PEPCK: Phosphoenolpyruvate carboxykinase; PKA: Protein kinase A; G6Pase: Glucose 6-phosphatase; PDE4B: Phosphodiesterase 4B; AMP: Activated protein kinase; LKB1: Liver kinase B1; MO-25: Mouse protein-25; Lep R: Leptin R; PP 2A: Protein phosphatase 2A; SREBPs: Sterol regulatory element-binding proteins; SCD1: Stearoyl-CoA desaturase-1; HMGR: HMG-CoA reductase = 3-hydroxy-3-methyl-glutaryl-CoA reductase; CPT1: Carnitine palmitoyl-transferase I; PFK2: Phosphofructokinase 2; PEPCK: Phosphoenolpyruvate carboxykinasem; mTOR: Mammalian target of rapamycin.

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Declaration

This plant is abundantly available in India, which has been collected and processed as per national guidelines.

Author contributions

VT and AT contributed to conceptualization and data curation; VT carried out formal analysis and investigation; VT provided methodology and software; AT and AS performed supervision; VT and AT performed validation and visualization; VT, AT, AS, MK, and NV contributed to roles/writing—original draft; VT, AT, AS, MK, NV, and AjS performed writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare no conflict of interest.

Competing interests

There is no conflict of interest regarding publication of the current research work.

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