Research Article

Bioassay-Guided Isolation, GC–MS And FT-IR Characterisation Of Antidiabetic Fractions From Portulaca Lutea Aerial Parts

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Abstract

Portulaca lutea (Portulacaceae) is an indigenous purslane used in folk practice for wounds and inflammatory skin conditions, yet its antidiabetic potential has not been systematically investigated. The present work reports successive extraction, phytochemical screening, acute toxicity, antidiabetic activity and in-vitro antioxidant evaluation of the aerial parts, followed by bioassay-guided isolation, GC–MS characterisation and FT-IR fingerprinting of the most active methanol fraction. Powdered aerial parts were extracted by Soxhlet with petroleum ether, chloroform, methanol and water in turn. Acute toxicity was assessed in mice and antidiabetic activity was examined at 200 mg/kg in Wistar rats using normoglycaemic, oral glucose tolerance, alloxan-induced (acute and 14-day) and streptozotocin-induced (acute and 14-day) models, with glibenclamide (10 mg/kg) as reference. Antioxidant activity of the methanol and aqueous extracts was measured by DPPH and superoxide scavenging and Folin–Ciocalteu total phenolics. The methanol extract (13.16 % w/w yield) contained alkaloids, glycosides, saponins, flavonoids, steroids/triterpenoids and tannins, and was non-toxic up to 2000 mg/kg. In alloxan-diabetic rats the methanol extract lowered blood glucose by 38.0 % at 8 h (single dose) and by 54.04 % on day 14 (multi-dose); in streptozotocin-diabetic rats the corresponding reductions were 37.83 % and 54.74 %. The methanol extract scavenged DPPH (IC50 19.20 µg/ml) and superoxide (IC50 34.41 µg/ml) and contained 65.33 mg GAE/g total phenolics. Column chromatography of the methanol extract on silica gel yielded four fractions (PL-M-01 to PL-M-04); GC–MS of PL-M-01 revealed triterpenoids and fatty acid esters, of PL-M-02 triterpenoids, of PL-M-03 flavonoids (luteolin, quercetin, kaempferol, apigenin, genistein), and of PL-M-04 alkaloids and catecholamines (dopamine, noradrenalin, oleraceins A–D). PL-M-01 and PL-M-03 at 50 mg/kg lowered blood glucose by 69 % and 62 % in alloxan rats and by 56 % and 54 % in STZ rats, respectively. FT-IR fingerprinting of PL-M-01 and PL-M-03 confirmed the functional groups assigned by GC–MS. The findings identify P. lutea methanol extract as a promising source of antidiabetic phenolics and triterpenoids suitable for further preclinical development.

Keywords: Portulaca lutea; Portulacaceae; antidiabetic activity; antioxidant; bioassay-guided fractionation; GC–MS; FT-IR; luteolin; catecholamines.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia resulting from impaired insulin secretion, insulin resistance, or a combination of the two, with associated glycosuria, hyperlipidaemia and ketonaemia [1,2]. The condition is classified as type 1 (insulin-dependent), type 2 (non-insulin-dependent) and a group of other specific forms, of which type 2 accounts for the vast majority of adult cases [3,4]. Existing oral hypoglycaemic agents — sulphonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors — provide symptomatic control but do not modify disease progression and carry characteristic adverse effects such as hypoglycaemia, lactic acidosis, weight gain and oedema [5,6,7]. Plants with credible ethnomedical records remain a productive starting point for new antidiabetic leads [8,9,10].

Oxidative stress is now recognised as a contributor to both the onset and the complications of diabetes: persistent hyperglycaemia promotes glucose auto-oxidation and generates reactive oxygen species that damage pancreatic β-cells, lipids and proteins [11,12]. Plant polyphenols and flavonoids with radical-scavenging capacity may buffer this damage and complement glucose-lowering therapy [13,14,15]. Several reviews have summarised the antidiabetic and antioxidant potential of medicinal plants [16,17,18,19,20,21,22,23,24].

The genus Portulaca (Portulacaceae) comprises about 40 species of succulent herbs. Portulaca oleracea L. (common purslane) is the most extensively studied species and is documented in the Materia Medica of Dioscorides, the Canon of Medicine of Avicenna and the Charaka Samhita for use against diarrhoea, throat infections, diabetes, obesity, toothache, asthma, ulcers, snake bites, jaundice and dysentery [25,26]. Pharmacological work on P. oleracea has

reported analgesic, anti-inflammatory, wound-healing, bronchodilatory, neuroprotective, hepatoprotective, hypocholesterolaemic and antidiabetic actions [27,28,29,30,31,32,33,34,35,36]. Phytochemical investigations of P. oleracea have identified omega-3 fatty acids, β-sitosterol and its glucoside, allantoin, the monoterpene glucoside portuloside A, the diterpene portulene, flavonoids (kaempferol, apigenin, myricetin, quercetin, luteolin) and alkaloids of the oleracein group (A–E) [37,38,39,40,41,42,43,44,45,46,47,48].

By contrast, the congener Portulaca lutea — known as the native yellow purslane or Indian purslane — has received little scientific attention despite its traditional use in wound healing, topical anti-inflammatory therapy and as an antioxidant [25,49]. The present work was therefore undertaken to evaluate successive petroleum ether, chloroform, methanol and aqueous extracts of P. lutea aerial parts for phytoconstituents, acute oral toxicity and antidiabetic activity in normoglycaemic, glucose-loaded, alloxan-induced and streptozotocin-induced rat models, in parallel with in-vitro antioxidant assays and total phenolic estimation. The most active methanol extract was then subjected to thin-layer and column chromatography, and the isolated fractions were characterised by GC–MS, screened for acute toxicity and tested for antidiabetic activity in alloxan- and streptozotocin-induced rats. The two most active fractions were further profiled by FT-IR. All data, tables and figures are drawn exclusively from the source thesis [50].

2. MATERIALS AND METHODS

2.1 Plant material

Whole aerial parts of P. lutea were collected in January 2018 from localities in Chennai and Bhubaneswar. The species was identified by Prof. V. Jayaraman, Presidency College, Chennai, and authenticated by Dr. P. C. Panda, Principal Scientist, Regional Plant Resource Centre, Bhubaneswar. A voucher specimen (V No. UIH-22549) was deposited in the herbarium of Hi-Tech College of Pharmacy, Bhubaneswar [50]. The material was washed, dried at 40 °C for 48 h and powdered with a mechanical grinder.

2.2 Chemicals and instruments

Alloxan monohydrate, streptozotocin, DPPH, Folin–Ciocalteu reagent, NBT, EDTA and ascorbic acid were from Sigma Chemical Co. (USA); glibenclamide from Aventis Pharma (Goa, India); solvents from Merck (Mumbai) and Qualigen (Mumbai); silica gel G (60–120 mesh) from Qualigen. GC–MS spectra were recorded on a Bruker DRX-500 with EIMS on a JEOL GC mate at the State Pollution Control Board, Odisha; elemental analysis on a Perkin-Elmer 2400 analyser; FT-IR spectra on a JASCO FT/IR-4600 type A spectrometer (serial E139561786, accessory ATR PRO ONE) at the School of Pharmaceutical Sciences, Siksha O Anusandhan (Deemed to be University). Blood glucose was measured with an Accu-Chek glucometer (Roche Diagnostics) using glucose oxidase–peroxidase strips.

2.3 Extraction

Five hundred grams of powdered aerial parts was packed in a Soxhlet apparatus and extracted successively with petroleum ether (PE), chloroform (CL) and methanol (ME), each for 24 h. The marc obtained after methanol extraction was heated with distilled water at 80 °C for 20 min to give the aqueous (AQ) extract. All extracts were concentrated under reduced pressure in a rotary evaporator and stored in a desiccator [51,52].

2.4 Preliminary phytochemical screening

Each extract was dispersed in water, heated gently, filtered, and the filtrate tested for alkaloids, glycosides, saponins, flavonoids, steroids/triterpenoids, tannins/polyphenolics and carbohydrates/reducing sugars using standard methods [53,54].

2.5 Acute oral toxicity

Albino mice (20–25 g) of either sex, housed at 25 ± 2 °C under a 12/12-h light–dark cycle with free access to standard pellets (Hindustan Lever, Kolkata) and water, were used. The protocol was approved by the Institutional Animal Ethics Committee of Hi-Tech Medical College & Hospital, Bhubaneswar (HMCH/IEC/9/12/19/IEC-D) and followed CPCSEA guidelines. Suspensions of each extract in 5 % Tween-80 were given by oral gavage at 500, 1000 and 2000 mg/kg using the up-and-down method [55,56]; LD50 was calculated by the method of Miller and Tainter [57] and one-tenth of the safe dose was used for screening [58].

2.6 Antidiabetic activity

Wistar albino rats (150–200 g) of either sex were divided into six groups of six each: Group 1 — solvent control (2 ml/kg); Group 2 — glibenclamide (10 mg/kg); Groups 3–6 — PE, CL, ME and AQ extracts (200 mg/kg) respectively, all by oral route. Five models were used: (i) normoglycaemic study with blood glucose measured at 0, 1, 2, 4, 8 and

24 h [59]; (ii) oral glucose tolerance test (OGTT) with glucose (2 g/kg, p.o.) given 30 min after the test article and blood glucose at 0, 30, 60, 90 and 120 min [60]; (iii) acute alloxan model — alloxan monohydrate (150 mg/kg, i.p.) in ice-cold saline, confirmation at 72 h, treatment with extract and sampling at 0, 1, 2, 4, 8 and 24 h [61]; (iv) 14-day alloxan model with daily dosing and sampling on days 0, 1, 2, 4, 7 and 14 [62]; (v) acute streptozotocin (STZ) model — STZ (65 mg/kg, i.p.) in citrate buffer pH 4.5 with 5 % glucose for 24 h to prevent STZ-induced hypoglycaemic mortality [63,64], confirmation at 72 h, and sampling at 0, 1, 2, 4, 8 and 24 h [65]; and (vi) 14-day STZ model with sampling on days 0, 1, 2, 4, 7 and 14 [66].

2.7 In-vitro antioxidant assays

DPPH radical scavenging was carried out with DPPH (0.004 % w/v in 95 % methanol) and extracts at 12.5, 25, 50, 75 and 100 µg/ml, with absorbance at 517 nm after 30 min [67,68]. Superoxide radical scavenging was measured using potassium superoxide in dry DMSO, NBT (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM), with absorbance at 560 nm after 150 min [69]. Ascorbic acid was the reference standard in both assays. Total phenolic content was estimated with Folin–Ciocalteu reagent at 760 nm using a gallic acid calibration curve and expressed as mg GAE/g of extract [70].

2.8 Chromatographic isolation

Thin-layer chromatography (TLC) was performed on silica gel G plates (5 × 20 cm, 0.25 mm thickness, activated at 110 °C for 1 h) developed in toluene : n-hexane : ethyl acetate : methanol (3 : 2 : 1 : 1) and visualised under UV light [71,72]. For column chromatography a glass column (500 × 40 mm) was packed with silica gel (60–120 mesh) by the wet-packing technique. The methanol extract (2 g) was adsorbed onto silica gel (80 g), evaporated to dryness, charged onto the column and eluted with the same solvent system; fractions of 20 ml were collected and monitored by TLC. Fractions with identical Rf values were pooled to give the isolated fractions PL-M-01 to PL-M-04 [73].

2.9 Characterisation of fractions

The four isolated fractions were characterised by GC–MS on a Bruker DRX-500 with EIMS recorded on a JEOL GC mate, with elemental analysis on a Perkin-Elmer 2400 analyser. Compounds were identified by comparison of retention times and mass fragmentation patterns with the NIST library. FT-IR spectra of the two most active fractions (PL-M-01 and PL-M-03) were recorded on a JASCO FT/IR-4600 spectrometer in KBr disc (1 % w/w, 8 MPa, 1 mm disk) over 7800.65–399.193 cm^{-1} at 21 °C and 50 % RH.

2.10 Fraction bioassay

Acute toxicity of PL-M-01 to PL-M-04 was assessed in mice by the up-and-down method as described above. Antidiabetic activity of the four fractions was tested at 50 mg/kg in alloxan- and streptozotocin-induced diabetic rats with the acute model described in Section 2.6 (iii) and (v).

2.11 Statistical analysis

All results are expressed as mean ± standard deviation (n = 6). Group differences were tested by one-way ANOVA followed by Dunnett's t-test, with $p < 0.05$ and $p < 0.01$ taken as significant.

3. RESULTS

3.1 Extractive yield and physical evaluation

Successive Soxhlet extraction of 500 g of aerial-part powder gave four extracts of distinct colour and consistency (Table 1). The methanol extract yielded the highest amount (13.16 % w/w), followed by the aqueous (9.32 %), petroleum ether (4.15 %) and chloroform (3.80 %) extracts. The methanol extract was dark brown and viscous, the aqueous extract light brown and viscous.

Table 1. Extractive values, colour and consistency of successive aerial-parts extracts of P. lutea.

Sl. No.	Type of extract	Colour	Appearance	% yield (w/w)
1	Petroleum ether (PE)	Pale yellow	Sticky, soft	4.15
2	Chloroform (CL)	Brown	Greasy, semi-solid	3.80
3	Methanol (ME)	Dark brown	Viscous	13.16
4	Aqueous (AQ)	Light brown	Viscous	9.32

3.2 Phytochemical screening

The methanol extract contained most of the tested phytochemicals (alkaloids, glycosides, saponins, flavonoids, steroids/triterpenoids, tannins/polyphenolics) except carbohydrates and amino acids. The aqueous extract was positive for alkaloids, glycosides, saponins, flavonoids, tannins and carbohydrates. The petroleum ether extract contained steroids/triterpenoids only, and the chloroform extract contained alkaloids, glycosides, flavonoids and steroids/triterpenoids (Table 2).

Table 2. Phytochemical screening of successive extracts of P. lutea aerial parts. (+) present, (–) absent.

Chemical group	PE	CL	ME	AQ
Alkaloids	–	+	+	+
Glycosides	–	+	+	+
Saponins	–	–	+	+
Flavonoids	–	+	+	+
Steroids / triterpenoids	+	+	+	–
Tannins / polyphenolics	–	–	+	+
Carbohydrate / reducing sugars	–	–	–	+
Amino acids / proteins	–	–	–	–

PE = petroleum ether, CL = chloroform, ME = methanol, AQ = aqueous.

3.3 Acute oral toxicity

No mortality and no gross behavioural change were recorded up to 2000 mg/kg for any extract; LD50 was therefore taken as greater than 2000 mg/kg, and 200 mg/kg was selected as the screening dose for the antidiabetic studies.

3.4 Antidiabetic activity

3.4.1 Normoglycaemic rats

None of the four extracts altered blood glucose in normoglycaemic rats over 8 h, with values remaining between 85.33 and 96.65 mg/dl. Glibenclamide lowered glucose from 87.85 to 56.64 mg/dl, a 35.52 % fall (Table 3, Fig. 1).

Table 3. Effect of P. lutea aerial-parts extracts on blood glucose levels (mg/dl) in normoglycaemic rats.

Group / treatment	0 h	1 h	2 h	4 h	8 h	F value
1 Control (2 ml/kg)	90.45±9.15	88.5±11.12	92.76±8.33	96.81±6.03	94.35±10.13	0.86
2 Glibenclamide (10 mg/kg)	87.85±7.22	73.98±8.16	65.33±8.37*	61.5±8.25*	56.64±9.32* (35.52)	46.24
3 PE (200 mg/kg)	89.56±5.29	94.33±5.16	90.45±4.77	92.65±5.59	96.15±3.18	0.37
4 CL (200 mg/kg)	85.33±6.88	90.86±4.53	83.25±4.97	93.66±5.29	90.5±4.19	0.68
5 ME (200 mg/kg)	89.83±5.11	87.65±5.69	85.16±5.52	82.25±5.11	88.45±4.92	1.30
6 AQ (200 mg/kg)	95.5±3.90	93.44±4.55	97.67±6.21	95.5±4.72	96.65±5.54	0.61

Values are mean ± SD (n = 6); one-way ANOVA followed by Dunnett's t-test; *p < 0.05, **p < 0.01. Figure in parenthesis indicates % fall in BGL versus 0 h.

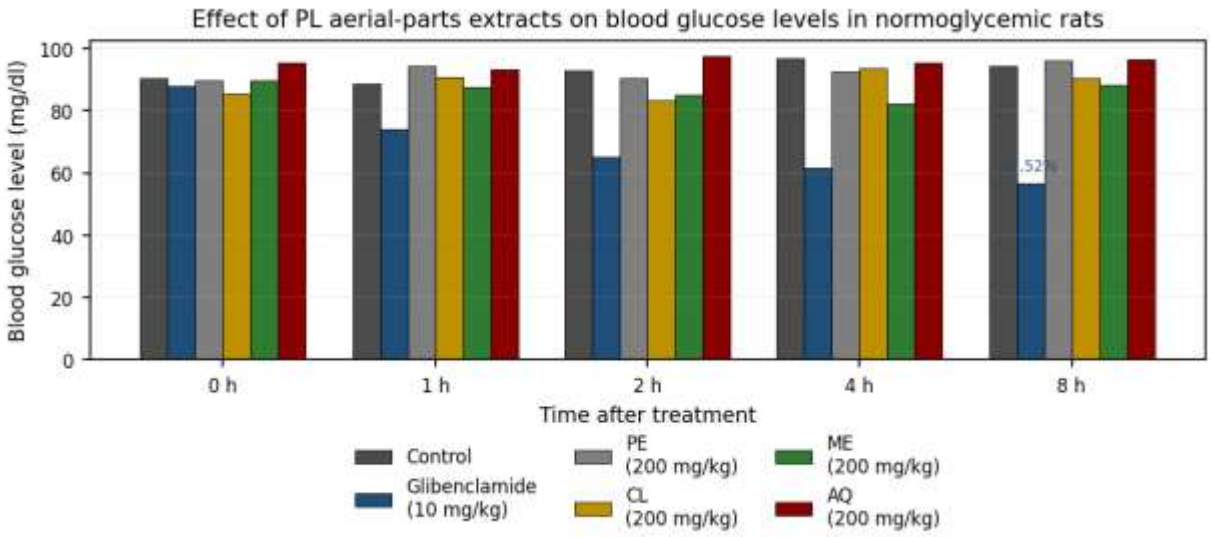

Fig. 1. Effect of P. lutea aerial-parts extracts on blood glucose levels in normoglycaemic rats.

3.4.2 Oral glucose tolerance test

Glucose loading raised BGL in all groups; the methanol extract produced a significant reduction ($p < 0.05$, $p < 0.01$) at 30, 60, 90 and 120 min, reaching 29.73 % fall at 120 min, while the aqueous extract reached 24.52 % and the chloroform extract 21.53 %. The petroleum ether extract was inactive. Glibenclamide produced a 32.10 % fall at 120 min (data from [50]).

3.4.3 Alloxan-induced diabetic rats (acute effect)

Single-dose treatment of alloxan-diabetic rats reduced BGL significantly ($p < 0.01$) from 2 h onward with the chloroform, methanol and aqueous extracts. The methanol extract lowered glucose from 254.46 to 157.75 mg/dl at 8 h (38.0 % fall), the aqueous extract from 241.78 to 168.44 mg/dl (30.33 % fall), and the chloroform extract from 237.35 to 201.87 mg/dl (14.95 % fall), while glibenclamide produced a 42.51 % fall. The petroleum ether extract was inactive (Table 4, Fig. 2).

Table 4. Effect of P. lutea aerial-parts extracts on blood glucose levels (mg/dl) in single-dose treated alloxan-induced diabetic rats.

Group / treatment	0 h	1 h	2 h	4 h	8 h	24 h	F
1 Control	232.16±8.68	235.84±17.62	238.50±8.98	244.86±17.38	247.66±15.50	253.75±16.08	0.44
2 Glibenclamide	242.66±5.78	234.5±7.47	209.33±8.43**	172.56±10.78**	139.5±4.16** (42.51)	240.83±3.65	210
3 PE	238.45±8.75	240±5.93	241.95±6.04	243.24±8.48	246.16±8.97	248.76±6.18	0.70
4 CL	237.35±7.03	234±5.09	226.5±5.28*	213±5.29**	201.87±6.30** (14.95)	239.27±6.28	25.15
5 ME	254.46±15.18	240.42±13.14	213.66±15.84**	173.16±13.16**	157.75±8.80** (38.0)	242.85±12.14	128.17
6 AQ	241.78±5.50	232.83±3.12	228**±4.51	205.5**±7.39	168.44±6.67** (30.33)	237.5±5.24	112.27

Values are mean ± SD (n = 6); *$p < 0.05$, **$p < 0.01$ versus solvent control. Figure in parenthesis indicates % fall in BGL versus 0 h.

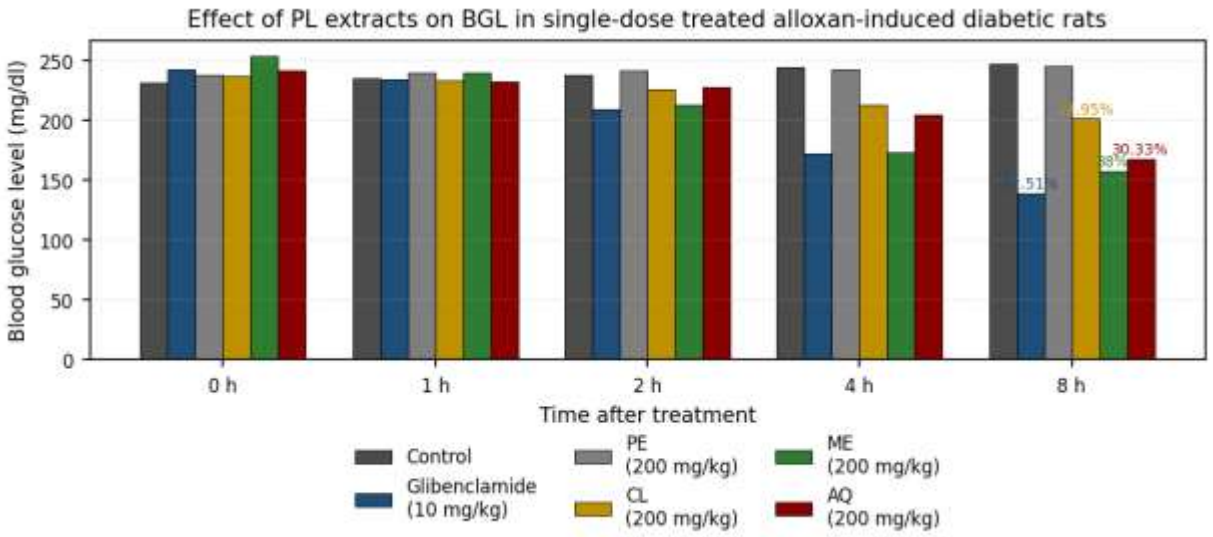

Fig. 2. Effect of P. lutea aerial-parts extracts on blood glucose levels in single-dose treated alloxan-induced diabetic rats.

3.4.4 Alloxan-induced diabetic rats (multi-dose, 14 days)

Daily treatment for 14 days progressively reduced BGL. The methanol extract lowered glucose by 54.04 % on day 14, the aqueous extract by 47.79 % and the chloroform extract by 34.36 %, against 64.75 % for glibenclamide. The petroleum ether and solvent-control groups showed a gradual rise (data from [50]).

3.4.5 Streptozotocin-induced diabetic rats (acute effect)

In the acute STZ model the methanol extract produced a 37.83 % fall in BGL at 8 h and the aqueous extract 26.14 %, against 43.30 % for glibenclamide; the chloroform extract gave a 19.68 % fall. Reductions were significant ($p < 0.01$) from 2 h onward for methanol and aqueous extracts (Table 5, Fig. 3).

Table 5. Effect of P. lutea aerial-parts extracts on blood glucose levels (mg/dl) in single-dose treated streptozotocin-induced diabetic rats.

Group / treatment	0 h	1 h	2 h	4 h	8 h	24 h	F
1 Control	237.20±6.55	242.65±8.16	249.33±10.46	251.74±7.16	254.13±9.51	257.26±7.44	0.96
2 Glibenclamide	241.66±7.02	233.16±6.99	207.35±7.42**	173.00±10.73**	137.25±8.00** (43.30)	242.16±4.26	223.10
3 PE	231.5±10.74	235.46±7.96	237.76±5.08	240.16±5.70	243.66±4.41	248.5±4.08	0.86
4 CL	234.5±11.09	230.33±8.71	221.83±5.03*	205.16±5.77**	188.33±6.18** (19.68)	229.16±5.03	28.84
5 ME	230.83±7.62	223.45±6.43	218.16±8.08**	188±6.63**	143.5±4.79** (37.83)	235.33±7.36	130.10
6 AQ	233.24±8.23	235±5.54	220.16±4.53**	197.16±3.54**	172.33±2.78** (26.14)	236.6±8.23	102.25

Values are mean ± SD (n = 6); *$p < 0.05$, **$p < 0.01$ versus solvent control. Figure in parenthesis indicates % fall in BGL versus 0 h.

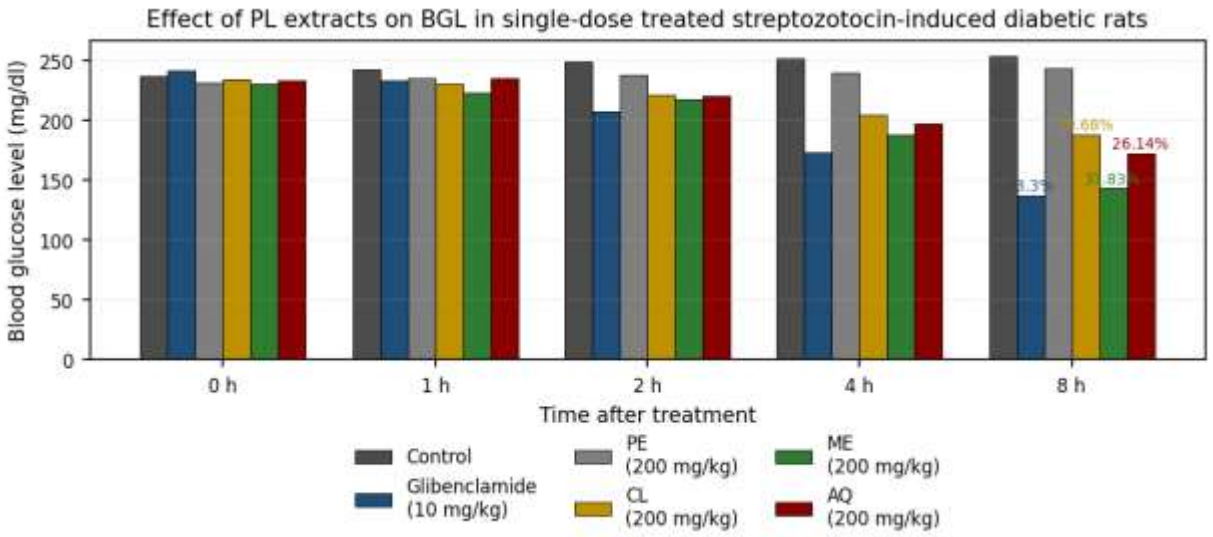

Fig. 3. Effect of P. lutea aerial-parts extracts on blood glucose levels in single-dose treated streptozotocin-induced diabetic rats.

3.4.6 Streptozotocin-induced diabetic rats (multi-dose, 14 days)

Daily treatment for 14 days gave a 54.74 % fall in BGL with the methanol extract, 49.10 % with the aqueous extract and 34.20 % with the chloroform extract, compared with 64.30 % for glibenclamide (Table 6, Fig. 4). Methanol and aqueous extracts were significant ($p < 0.01$) from day 1 onward; the chloroform extract was significant ($p < 0.01$) from day 2.

Table 6. Effect of P. lutea aerial-parts extracts on blood glucose levels (mg/dl) in multi-dose treated streptozotocin-induced diabetic rats.

Group / treatment	Day 0	Day 1	Day 2	Day 4	Day 7	Day 14	F
1 Control	235.83±9.36	238.25±7.47	242.5±6.11	247.25±10.08	250.45±6.98	255.5±7.81	0.74
2 Glibenclamide	237.45±5.19	218.36±6.53**	191.85±6.75**	157.16±6.43**	131.33±3.98**	84.75±3.86** (64.30)	627
3 PE	228.5±8.08	235.16±4.07	238.8±5.67	242.5±4.88	246.25±6.67	251.5±6.65	0.89
4 CL	235.83±7.62	229.77±6.73	214.5±7.28**	195.66±7.44**	177±9.16**	155.16±9.02** (34.20)	92.76
5 ME	232.35±9.28	221.83±9.94**	202.16±11.19**	183±11.08**	160.16±8.72**	105.15±4.35** (54.74)	141
6 AQ	236.5±7.41	228.16±7.46**	207.66±6.58**	190.5±6.24**	166.72±4.17**	121.36±3.36** (49.10)	270.86

Values are mean ± SD (n = 6); *$p < 0.05$, **$p < 0.01$ versus solvent control. Figure in parenthesis indicates % fall in BGL versus day 0.

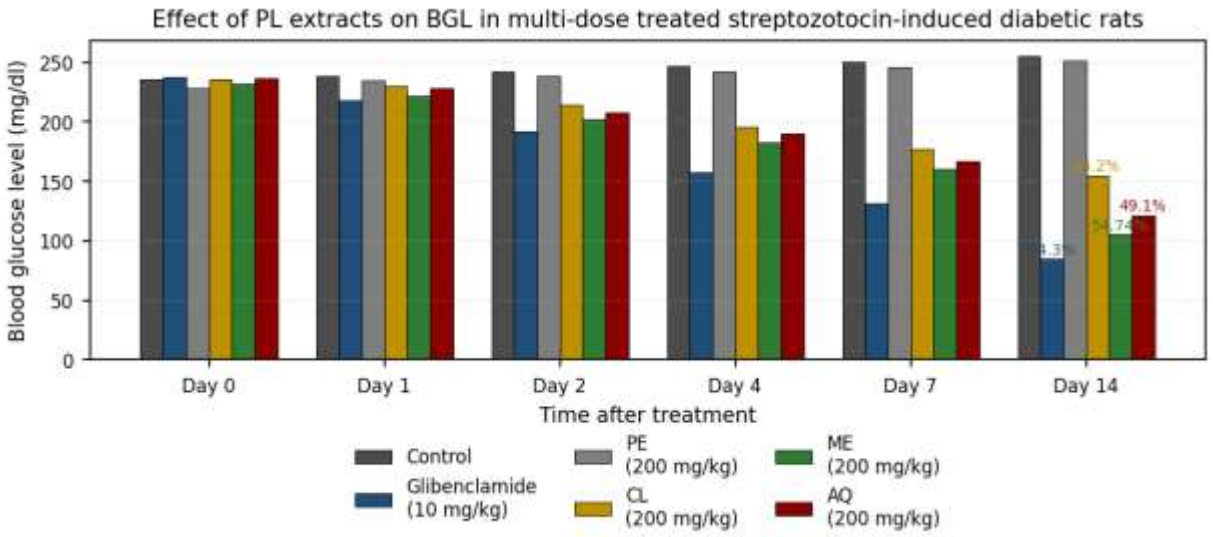

Fig. 4. Effect of P. lutea aerial-parts extracts on blood glucose levels in multi-dose treated streptozotocin-induced diabetic rats.

3.5 Antioxidant activity

Both methanol and aqueous extracts scavenged DPPH and superoxide radicals in a concentration-dependent fashion. The methanol extract inhibited DPPH by 76.41 % at 100 µg/ml (IC50 19.20 µg/ml), the aqueous extract by 72.13 % (IC50 34.21 µg/ml), against 83.93 % (IC50 8.08 µg/ml) for ascorbic acid (Tables 7–9, Fig. 5). For superoxide, the methanol extract reached 66.90 % inhibition at 100 µg/ml (IC50 34.41 µg/ml), the aqueous extract 62.18 % (IC50 54.37 µg/ml), against 77.93 % (IC50 11.37 µg/ml) for ascorbic acid (Tables 10–12, Fig. 6).

Table 7. DPPH radical scavenging activity of ascorbic acid.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
Ascorbic acid	12.5	0.416 ± 0.003	51.90 ± 0.32	8.08
	25	0.375 ± 0.011	56.64 ± 0.86	
	50	0.307 ± 0.006	64.50 ± 0.25	
	75	0.206 ± 0.012	76.18 ± 0.98	
	100	0.139 ± 0.014	83.93 ± 0.59	

Table 8. DPPH radical scavenging activity of the methanol extract of P. lutea.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
ME of PL	12.5	0.452 ± 0.017	47.74 ± 1.55	19.20
	25	0.416 ± 0.032	51.90 ± 0.91	
	50	0.338 ± 0.009	60.92 ± 0.49	
	75	0.298 ± 0.011	65.54 ± 1.16	
	100	0.204 ± 0.018	76.41 ± 1.65	

ME = methanol extract; PL = Portulaca lutea.

Table 9. DPPH radical scavenging activity of the aqueous extract of P. lutea.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
AQ of PL	12.5	0.496 ± 0.026	42.65 ± 0.92	34.21
	25	0.452 ± 0.041	47.74 ± 1.45	
	50	0.395 ± 0.014	54.33 ± 2.12	
	75	0.314 ± 0.008	63.69 ± 1.16	
	100	0.241 ± 0.019	72.13 ± 0.96	

AQ = aqueous extract.

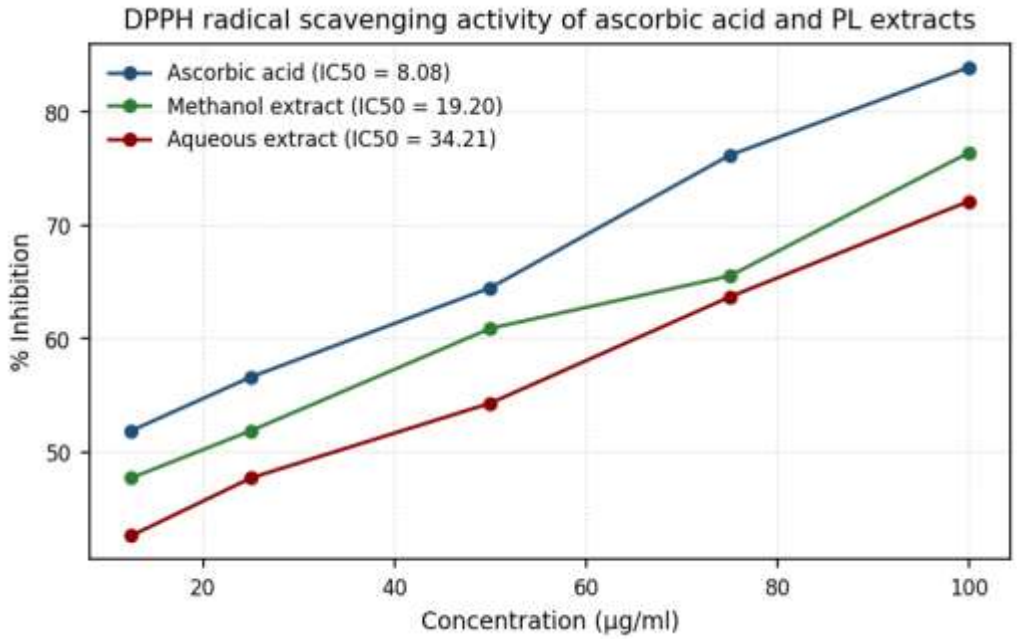

Fig. 5. DPPH radical scavenging activity of ascorbic acid, methanol and aqueous extracts of P. lutea.

Table 10. Superoxide radical scavenging activity of ascorbic acid.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
Ascorbic acid	12.5	0.415 ± 0.013	49.69 ± 1.12	11.37
	25	0.376 ± 0.017	54.42 ± 0.68	
	50	0.301 ± 0.008	63.51 ± 0.89	
	75	0.242 ± 0.023	70.66 ± 0.78	
	100	0.182 ± 0.006	77.93 ± 1.15	

Table 11. Superoxide radical scavenging activity of the methanol extract of P. lutea.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
ME of PL	12.5	—	40.24	34.41
	25	—	50.42	
	50	—	55.51	
	75	—	62.66	
	100	—	66.90	

Absorbance values not tabulated in the source thesis [50]; % inhibition values transcribed as reported.

Table 12. Superoxide radical scavenging activity of the aqueous extract of P. lutea.

Test material	Conc. (µg/ml)	Absorbance	% Inhibition	IC50 (µg/ml)
AQ of PL	12.5	0.521 ± 0.016	36.84 ± 1.67	54.37
	25	0.478 ± 0.085	42.31 ± 2.88	
	50	0.408 ± 0.009	50.54 ± 1.52	
	75	0.365 ± 0.054	55.75 ± 1.08	
	100	0.312 ± 0.071	62.18 ± 1.02	

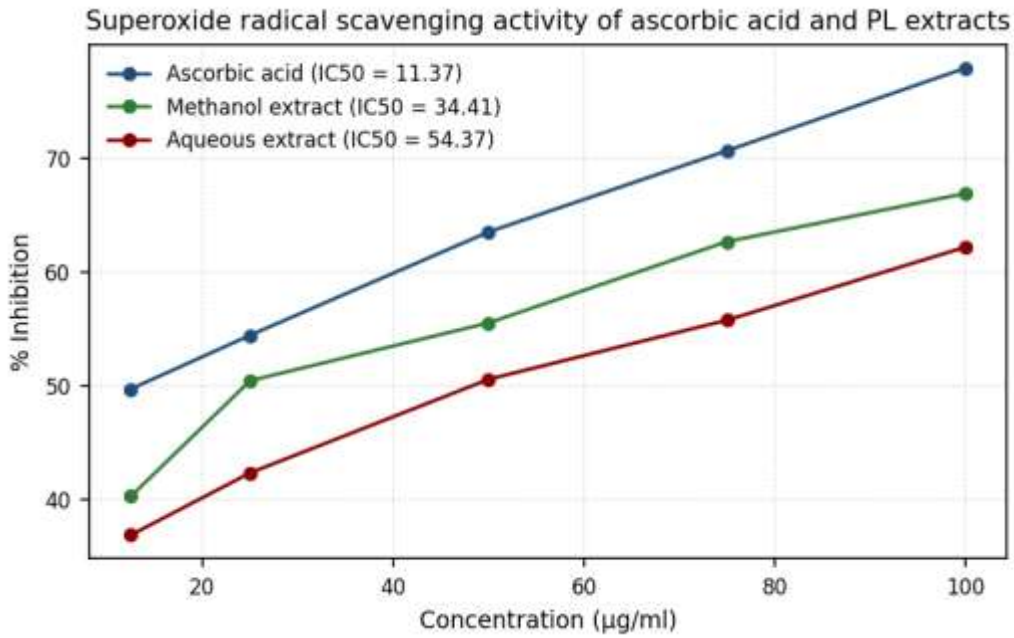

Fig. 6. Superoxide radical scavenging activity of ascorbic acid, methanol and aqueous extracts of P. lutea.

3.6 Total phenolic content

The methanol extract contained 65.33 ± 1.88 mg GAE/g of total phenolics, approximately 3.5 times the level of the aqueous extract (18.66 ± 1.03 mg GAE/g), calculated from the gallic acid calibration curve y = 0.015x + 0.025 (R^2 = 0.998) (Table 13, Fig. 7).

Table 13. Total phenolic content of methanol and aqueous extracts of P. lutea.

Test extract	Conc. (µg/ml)	Absorbance	Total phenolics (mg GAE/g)
Methanol extract	100	0.0348 ± 0.0031	65.33 ± 1.88
Aqueous extract	100	0.0276 ± 0.0182	18.66 ± 1.03

GAE = gallic acid equivalent.

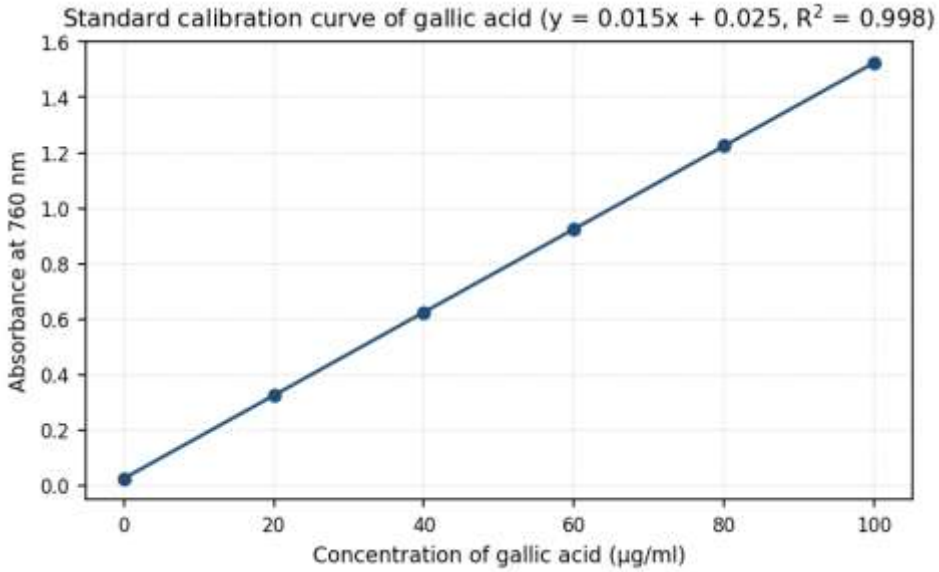

Fig. 7. Standard calibration curve of gallic acid used for total phenolic estimation.

3.7 Chromatographic isolation

TLC of the methanol extract on silica gel G in toluene : n-hexane : ethyl acetate : methanol (3 : 2 : 1 : 1) gave well-resolved spots under UV light. Column chromatography on silica gel (60–120 mesh) with the same solvent system gave five fractions, of which four were obtained in usable quantity; the fifth was discarded due to negligible mass. The four isolated fractions were coded PL-M-01, PL-M-02, PL-M-03 and PL-M-04 (Table 14).

Table 14. Rf values of compounds isolated from the methanol extract of P. lutea by column chromatography (TLC solvent toluene : n-hexane : ethyl acetate : methanol 3 : 2 : 1 : 1).

Fraction code	No. of TLC spots	Colour of fraction	Rf value(s)
PL-M-01	1	Colourless	0.72
PL-M-02	2	White	0.67 & 0.58
PL-M-03	1	Light yellowish	0.57
PL-M-04	1	Brownish yellow	0.51
(PL-M-05)	1	Deep brown	0.40 (not taken — negligible mass)

3.8 Phytoconstituents of isolated fractions

Preliminary phytochemical tests showed that flavonoids and glycosides were present in PL-M-03, alkaloids in PL-M-04, sterols in PL-M-01, PL-M-02 and PL-M-03, and terpenoids in PL-M-01 and PL-M-02 (Table 15).

Table 15. Phytoconstituents present in isolated fractions of P. lutea methanol extract. (+) present, (–) absent.

Phytoconstituents	PL-M-01	PL-M-02	PL-M-03	PL-M-04
Flavonoids	–	–	++	–
Alkaloids	–	–	–	+
Glycosides	–	–	+	–
Sterols	+	+	+	–
Terpenoids	+	+	–	–
Tannins	–	–	–	–

3.9 GC–MS characterisation of fractions

GC–MS analysis of the four fractions identified 13 compounds in PL-M-01, 8 in PL-M-02, 12 in PL-M-03 and 14 in PL-M-04. The major compound classes were: PL-M-01 — higher fatty acids, fatty acid esters and triterpenoids (Nonanedioic acid dimethyl ester; 1,2,4-Trioxolane-2-octanoic acid 5-octyl- methyl ester; tetradecanoic acid; 9-octadecenoic acid (Z)- phenylmethyl ester; (3β,5α)-cholestan-3-ol 2-methylene; heptadecanoic acid 16-methyl-methyl ester; 9,12,15-octadecatrienoic acid 2,3-dihydroxypropyl ester (Z,Z,Z)-; hexadecanoic acid 14-methyl- methyl ester; oleic acid 3-(octadecyloxy)propyl ester; lupeol; 24-nor-cholest-22-ene; sitostenone; 9-hexadecenoic acid 9-octadecenyl ester (Z,Z)-); PL-M-02 — triterpenoids (ethanol 2-(octadecyloxy)-; stigmastanol; phytyl palmitate; phytyl dodecanoate; tetracosanoic acid methyl ester; daucosterol; portuloside A; friedelane); PL-M-03 — flavonoids (methyl palmitate; linoleic acid; 6,9,12,15-docosatetraenoic acid methyl ester; catechol; desulphosinigrin; genistein; apigenin; kaempferol; luteolin; quercetin; calcitriol; 1-monolinoleoylglycerol trimethylsilyl ether); PL-M-04 — alkaloids and catecholamines (dopamine; noradrenalin; indole-3-carboxylic acid; 3-quinolinecarboxylic acid; cyclo(L-tyrosinyl-L-tyrosine); n-cis-feruloyltyramine; lonchocarpic acid; linoleic acid; eicosapentaenoic acid; aurantiamide; oleraceins A, B, C and D) (Tables 16–19).

Table 16. Compounds identified by GC–MS in fraction PL-M-01 of P. lutea methanol extract.

Sl. No.	Compound identified in PL-M-01	Class
1	Nonanedioic acid, dimethyl ester	Fatty acid ester
2	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	Fatty acid ester
3	Tetradecanoic acid	Fatty acid
4	9-Octadecenoic acid (Z)-, phenylmethyl ester	Fatty acid ester
5	(3β,5α)-Cholestan-3-ol, 2-methylene	Triterpenoid

6	Heptadecanoic acid, 16-methyl-, methyl ester	Fatty acid ester
7	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z)-	Fatty acid ester
8	Hexadecanoic acid, 14-methyl-, methyl ester	Fatty acid ester
9	Oleic acid, 3-(octadecyloxy)propyl ester	Fatty acid ester
10	Lupeol	Triterpenoid
11	24-nor-Cholest-22-ene	Triterpenoid
12	Sitostenone	Triterpenoid / sterol
13	9-Hexadecenoic acid, 9-octadecenyl ester (Z,Z)-	Fatty acid ester

Table 17. Compounds identified by GC–MS in fraction PL-M-02 of P. lutea methanol extract.

Sl. No.	Compound identified in PL-M-02	Class
1	Ethanol, 2-(octadecyloxy)-	Fatty alcohol
2	Stigmastanol	Triterpenoid / sterol
3	Phytyl palmitate	Triterpenoid ester
4	Phytyl dodecanoate	Triterpenoid ester
5	Tetracosanoic acid, methyl ester	Fatty acid ester
6	Daucosterol	Sterol glycoside
7	Portuloside A	Monoterpene glucoside
8	Friedelane	Triterpenoid

Table 18. Compounds identified by GC–MS in fraction PL-M-03 of P. lutea methanol extract.

Sl. No.	Compound identified in PL-M-03	Class
1	Methyl palmitate	Fatty acid ester
2	Linoleic acid	Fatty acid
3	6,9,12,15-Docosatetraenoic acid, methyl ester	Fatty acid ester
4	Catechol	Phenolic
5	Desulphosinigrin	Glycoside
6	Genistein	Isoflavone
7	Apigenin	Flavone
8	Kaempferol	Flavonol
9	Luteolin	Flavone
10	Quercetin	Flavonol
11	Calcitriol	Secosteroid
12	1-Monolinoleoylglycerol trimethylsilyl ether	Glyceroester

Table 19. Compounds identified by GC–MS in fraction PL-M-04 of P. lutea methanol extract.

Sl. No.	Compound identified in PL-M-04	Class
1	Dopamine	Catecholamine alkaloid
2	Noradrenalin	Catecholamine alkaloid
3	Indole-3-carboxylic acid	Indole alkaloid
4	3-Quinolinecarboxylic acid	Quinoline alkaloid
5	Cyclo(L-tyrosinyl-L-tyrosine)	Cyclic dipeptide
6	n-cis-Feruloyltyramine	Phenolic amide
7	Lonchocarpic acid	Fatty acid
8	Linoleic acid	Fatty acid
9	Eicosapentaenoic acid	Fatty acid
10	Aurantiamide	Peptide alkaloid
11	Oleracein A	Betaxanthin alkaloid

12	Oleracein B	Betaxanthin alkaloid
13	Oleracein C	Betaxanthin alkaloid
14	Oleracein D	Betaxanthin alkaloid

3.10 Acute toxicity of fractions

No mortality and no significant gross behavioural change were observed at 500 mg/kg for any of the four fractions. The screening dose of 50 mg/kg was therefore selected for the antidiabetic bioassay.

3.11 Antidiabetic activity of isolated fractions

In the acute alloxan model, PL-M-01 lowered BGL from 241.35 to 172.26 mg/dl at 8 h (69 % fall), and PL-M-03 from 243.46 to 181.75 mg/dl (62 % fall), while glibenclamide achieved a 71 % fall. PL-M-02 and PL-M-04 were markedly less active (16 % and 30 % fall respectively) (Table 20, Fig. 8). In the acute STZ model, PL-M-01 lowered BGL from 243.05 to 187.66 mg/dl (56 % fall) and PL-M-03 from 239.83 to 185.65 mg/dl (54 % fall), against 84 % for glibenclamide; PL-M-02 and PL-M-04 gave 36 % and 34 % falls respectively (Table 21, Fig. 9). PL-M-01 and PL-M-03 were significant ($p < 0.01$) from 2 h onward in both models.

Table 20. Effect of isolated fractions of P. lutea methanol extract on blood glucose levels (mg/dl) in single-dose treated alloxan-induced diabetic rats.

Group / treatment	0 h	1 h	2 h	4 h	8 h	24 h	F
Control (2 ml/kg)	231.14±8.68	232.54±17.62	239.52±8.98	267.46±17.38	241.44±15.50	255.95±16.08	0.43
Glibenclamide (10 mg/kg)	238.25±5.78	233.1±7.47	201.21±8.43**	171.36±10.78**	167.5±4.16** (71)	237.53±3.65	208
PL-M-01 (50 mg/kg)	241.35±8.75	239.87±5.93	220.35±6.04	203.27±8.48	172.26±8.80** (69)	241.16±6.18	113.56
PL-M-02 (50 mg/kg)	255.35±7.03	250.43±5.09	246.5±5.28*	241.21±5.29**	239.87±6.30** (16)	259.27±6.28	25.15
PL-M-03 (50 mg/kg)	243.46±15.18	241.42±13.14	223.66±15.84**	199.16±13.16**	181.75±8.97** (62)	242.85±12.14	118.17
PL-M-04 (50 mg/kg)	252.78±5.50	257.83±3.12	249.77±4.51**	238.15±7.39**	225.44±6.67** (30)	261.5±5.24	29.27

Values are mean ± SD (n = 6); *p < 0.05, **p < 0.01 versus solvent control. Figure in parenthesis indicates % fall in BGL versus 0 h.

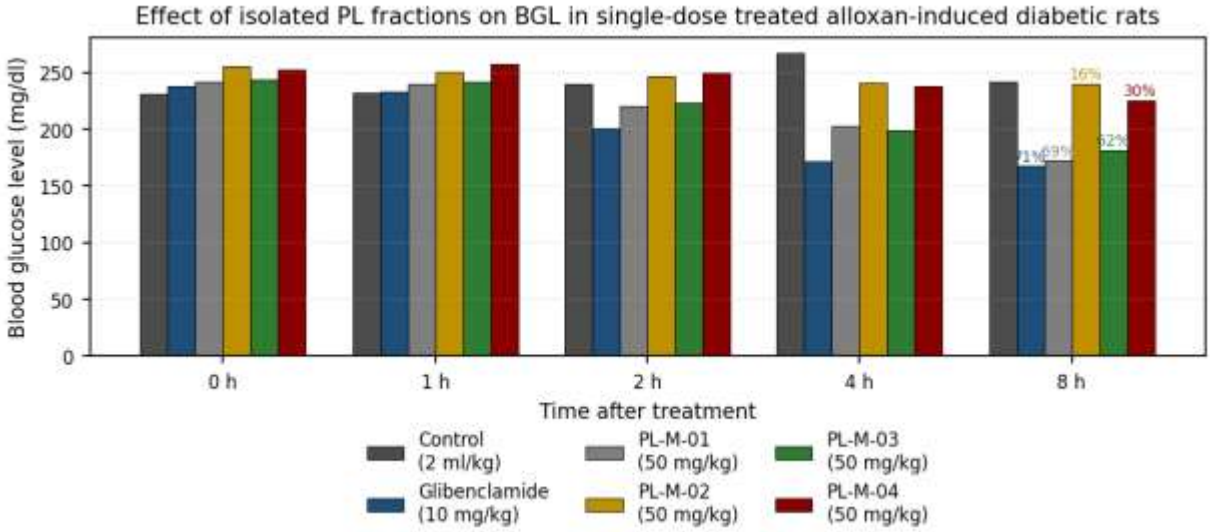

Fig. 8. Effect of isolated fractions of P. lutea methanol extract on blood glucose levels in single-dose treated alloxan-induced diabetic rats.

Table 21. Effect of isolated fractions of P. lutea methanol extract on blood glucose levels (mg/dl) in single-dose treated streptozotocin-induced diabetic rats.

Group / treatment	0 h	1 h	2 h	4 h	8 h	24 h	F
Control (2 ml/kg)	237.20±6.55	242.65±8.16	249.33±10.46	251.74±7.16	254.13±9.51	257.26±7.44	0.96
Glibenclamide (10 mg/kg)	241.66±7.02	233.16±6.99	207.35±7.42**	173.00±10.73**	157.25±8.00** (84)	242.16±4.26	223.10
PL-M-01 (50 mg/kg)	243.05±10.74	239.46±7.96	221.76±5.08	206.16±5.70	187.66±4.41** (56)	249.5±4.08	130.86
PL-M-02 (50 mg/kg)	234.5±11.09	232.33±8.71	229.83±5.03*	215.16±5.77**	198.33±6.18** (36)	259.16±5.03	28.84
PL-M-03 (50 mg/kg)	239.83±7.62	238.45±6.43	222.16±8.08**	209.08±6.63**	185.65±4.79** (54)	245.33±7.36	127.10
PL-M-04 (50 mg/kg)	233.24±8.23	235.27±5.54	225.16±4.53**	217.16±3.54**	199.33±2.78** (34)	260.6±8.23	32.25

Values are mean ± SD (n = 6); *p < 0.05, **p < 0.01 versus solvent control. Figure in parenthesis indicates % fall in BGL versus 0 h.

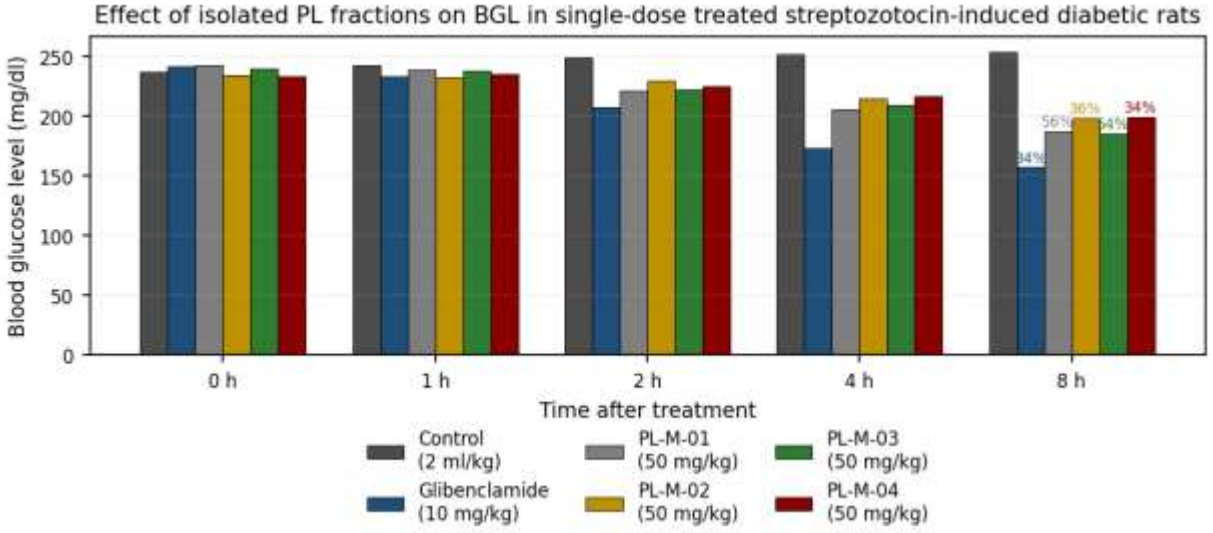

Fig. 9. Effect of isolated fractions of P. lutea methanol extract on blood glucose levels in single-dose treated streptozotocin-induced diabetic rats.

3.12 FT-IR fingerprinting

FT-IR spectra of PL-M-01 and PL-M-03 were recorded over 7800.65–399.193 cm^{-1}. The broad bands at 3360.20–3362.20 cm^{-1} correspond to free hydroxyl (–OH, Ar–OH) and amine/amide (N–H) stretching of alcohols, organic acids, phenols and proteins, consistent with phytols, stigmasterol, inositol, tyrosinol, lupeol, lanosterol, tocopherols, cholesterol, glycerin, eicosanol, octadecanamide, oleic acid amide, luteolin and docosenamide. Smaller peaks at 2979.65–2834.99 cm^{-1} arise from C–C, C=C and C≡C stretching of alkanes, alkenes and alkynes of fatty acids and lipids (neophytadiene, squalene, amyrin, norursadiene, hexamethyldodecane, nonadecene and tetrapentacontane). The broad array at 1651.10–1104.41 cm^{-1} corresponds to the carbonyl (C=O) of esters and ketones, carboxylic acids, aldehydes, aliphatic amines (C–N), phosphoryl (P=O) and nitro (–NO) groups, and nucleic acids (cyclopentadione, octadecanoic acid, methyl commate-D, sitostenone and guanosine). Lower-intensity peaks at 1019.17–1019.90 cm^{-1} indicate aryl and ether C–O–C stretching and di-/tri-substituted aromatics (pyrrolidine and related) (Table 22).

Table 22. FT-IR band assignments for fractions PL-M-01 and PL-M-03 of P. lutea methanol extract (recorded on JASCO FT/IR-4600, KBr disc, 7800.65–399.193 cm^{-1}).

Wavenumber range (cm^{-1})	Functional group	Indicative phytoconstituents
3360.20 – 3362.20	Free –OH (Ar–OH) and N–H stretching (alcohols, organic acids, phenols, amine/amide of proteins and water)	Phytols, stigmasterol, inositol, tyrosinol, lupeol, lanosterol, tocopherols, cholesterol, glycerin, eicosanol, octadecanamide, oleic acid amide, luteolin, docosenamide
2979.65 – 2834.99	C–C, C=C, C≡C stretching of alkanes, alkenes and alkynes of fatty acids and lipids	Neophytadiene, squalene, amyrin, norursadiene, hexamethyldodecane, nonadecene, tetrapentacontane
1651.10 – 1104.41	Carbonyl (C=O) of esters, ketones, carboxylic acids, aldehydes; aliphatic amines (C–N); phosphoryl (P=O); nitro (–NO); nucleic acids	Cyclopentadione, octadecanoic acid, methyl commate-D, sitostenone, guanosine
1019.17 – 1019.90	Aryl and ether C–O–C; di- and tri-substituted aromatic C=C	Pyrrolidine and related heterocycles

4. DISCUSSION

Successive Soxhlet extraction with solvents of increasing polarity concentrated the polar constituents of P. lutea aerial parts into the methanol and aqueous extracts, which together accounted for 22.5 % of the dry weight. Phytochemical screening confirmed that alkaloids, glycosides, saponins, flavonoids, steroids/triterpenoids and tannins were preferentially recovered in these polar fractions. The high yield and rich phytochemical profile of the methanol extract are in line with earlier reports that alcoholic solvents are more effective than water or non-polar solvents for recovering bioactive polar constituents from plant material [25]. The absence of mortality at 2000 mg/kg indicates a wide safety margin and supports 200 mg/kg as the screening dose for antidiabetic testing.

In normoglycaemic rats none of the extracts lowered glucose below the baseline, indicating that they do not behave as insulin or sulphonylurea mimetics in the normal state [74,75]. In the oral glucose tolerance test, in contrast, the methanol and aqueous extracts restrained the post-prandial rise in glucose by 29.73 % and 24.52 % respectively at 120 min, comparable in pattern with glibenclamide (32.10 %). This points to an insulin-independent mechanism, most likely inhibition of endogenous glucose production or of intestinal glucose absorption [76,77], and is consistent with the absence of hypoglycaemia in normoglycaemic animals.

Both acute and 14-day studies in alloxan- and streptozotocin-induced diabetic rats confirmed the superiority of the methanol extract over the aqueous and chloroform extracts, and the inactivity of the petroleum ether fraction. Alloxan destroys pancreatic β-cells through the generation of reactive oxygen species during redox cycling in the presence of reducing agents such as glutathione and cysteine [78,79]; the demonstrable glucose-lowering effect of the methanol extract in this model therefore implies either residual β-cell stimulation or extra-pancreatic action. STZ produces diabetes by alkylating β-cell DNA and depleting NAD^+, leading to progressive insulin deficiency [80,81]. The fact that the methanol extract lowered glucose by 37.83 % at 8 h and 54.74 % at day 14 in STZ rats — a model with severe β-cell loss — favours an extra-pancreatic component, namely enhanced peripheral glucose disposal or inhibition of hepatic gluconeogenesis [82,83,84,85]. The persistence of the effect across 14 days indicates that the active principles remain available with repeated dosing and may support residual β-cell recovery [86].

Flavonoids, sterols, alkaloids, saponins and polyphenolic compounds are well documented bioactive antidiabetic principles [87,88,89,90,91], and β-cell regeneration by certain flavonoids has been reported [92]. Since the methanol and aqueous extracts of P. lutea contained several of these classes, the observed activity is plausibly attributable to a combination of constituents rather than to a single compound. The antioxidant data align with this picture: the methanol extract, with IC50 of 19.20 µg/ml against DPPH and 34.41 µg/ml against superoxide, was clearly more potent than the aqueous extract (34.21 and 54.37 µg/ml), and ranked with its higher total phenolic content (65.33 versus 18.66 mg GAE/g). Polyphenols act as primary antioxidants through hydrogen donation and singlet-oxygen quenching [93,94,95], and their redox behaviour is widely regarded as the principal driver of the radical-scavenging

activity of plant extracts [96]. The lower IC50 of the methanol extract therefore supports the view that phenolics contribute both to antioxidant and to glucose-lowering action, the latter partly through protection of pancreatic β-cells from oxidative damage [97,98,99].

Because the methanol extract outperformed all other extracts of P. lutea and also compared favourably with the methanol extract of C. rutidosperma studied in parallel [50], it was selected for chromatographic fractionation. TLC followed by column chromatography on silica gel gave four usable fractions, PL-M-01 to PL-M-04, with distinct Rf values (0.51–0.72). GC–MS assigned the major compound classes to each fraction: triterpenoids and fatty acid esters in PL-M-01; triterpenoids in PL-M-02; flavonoids (genistein, apigenin, kaempferol, luteolin, quercetin) in PL-M-03; and alkaloids and catecholamines (dopamine, noradrenalin, oleraceins A–D) in PL-M-04. Several of these compounds have established antidiabetic and antioxidant credentials: luteolin and quercetin inhibit α-glucosidase and protect β-cells [100,101]; the oleraceins are nitrogenous pigments unique to Portulaca with reported antioxidant action [46]; lupeol and β-sitosterol modulate glucose metabolism in animal models [102,103]; and the catecholamines identified in PL-M-04 confirm the earlier detection of L-noradrenalin in P. oleracea [37].

The bioassay-guided screen identified PL-M-01 and PL-M-03 as the most active fractions, lowering BGL by 69 % and 62 % in alloxan-diabetic rats and by 56 % and 54 % in STZ-diabetic rats at 8 h. PL-M-01 contains triterpenoids (lupeol, sitostenone, 24-nor-cholest-22-ene) and long-chain fatty acid esters, a profile consistent with the well-documented antidiabetic activity of plant sterols and triterpenes [87,102,103]. PL-M-03 contains the flavonoid cluster luteolin, quercetin, kaempferol, apigenin and genistein, all of which have been shown to inhibit carbohydrate-hydrolysing enzymes, protect β-cells from oxidative damage and enhance peripheral glucose uptake [100,101,104]. FT-IR fingerprinting of PL-M-01 and PL-M-03 confirmed the functional groups assigned by GC–MS: hydroxyl/phenolic O–H and amine N–H stretching at 3360 cm^{-1}, aliphatic C–H stretching at 2979–2834 cm^{-1}, carbonyl absorption at 1651–1104 cm^{-1} consistent with esters of fatty acids and triterpenoids, and aryl/ether C–O–C stretching near 1019 cm^{-1} characteristic of flavonoid aglycones. The convergence of GC–MS and FT-IR evidence therefore identifies the flavonoid cluster of PL-M-03 and the triterpenoid/sterol cluster of PL-M-01 as the principal carriers of the antidiabetic activity of P. lutea methanol extract.

These findings carry two practical implications. First, they support the ethnomedical use of P. lutea aerial parts as a source of antidiabetic and antioxidant compounds and identify the methanol extract as the optimal starting material for further development. Second, they indicate that the antidiabetic action of P. lutea is shared between two chemically distinct clusters — triterpenoid/sterol esters and flavonoid aglycones — and that bioassay-guided work should target both. Further studies should quantify the active constituents, evaluate their pharmacokinetics, elucidate their molecular targets and confirm long-term safety.

5. CONCLUSION

Successive Soxhlet extraction of P. lutea aerial parts gave four extracts of which the methanol fraction showed the highest yield (13.16 % w/w), the richest phytochemical profile and the strongest antidiabetic and antioxidant activity. At 200 mg/kg the methanol extract produced significant ($p < 0.01$) blood-glucose lowering in alloxan- and streptozotocin-induced diabetic rats under both acute and 14-day regimens, with a maximum 54.74 % reduction in the STZ multi-dose model. The same extract scavenged DPPH and superoxide radicals with IC50 values of 19.20 and 34.41 µg/ml and contained 65.33 mg GAE/g of total phenolics. Column chromatography of the methanol extract yielded four fractions, of which PL-M-01 (triterpenoid/sterol/fatty acid ester cluster) and PL-M-03 (flavonoid cluster: luteolin, quercetin, kaempferol, apigenin, genistein) showed the strongest antidiabetic activity at 50 mg/kg. FT-IR fingerprinting of PL-M-01 and PL-M-03 confirmed the functional groups assigned by GC–MS. The work identifies P. lutea as a promising source of antidiabetic phenolics and triterpenoids suitable for further preclinical development.

List of Symbols and Abbreviations

ANOVA — analysis of variance
AQ — aqueous extract
BGL — blood glucose level
CL — chloroform extract
DMSO — dimethyl sulfoxide
DPPH — 2,2-diphenyl-1-picrylhydrazyl
EDTA — ethylenediaminetetraacetic acid
EIMS — electron-impact mass spectrometry

F — F-statistic from ANOVA
FT-IR — Fourier-transform infrared (spectroscopy)
GAE — gallic acid equivalent
GC–MS — gas chromatography–mass spectrometry
i.p. — intraperitoneal
ME — methanol extract
NBT — nitroblue tetrazolium
OGTT — oral glucose tolerance test
p.o. — per oral
PE — petroleum ether extract
PL — Portulaca lutea
PL-M-01…04 — isolated methanol-extract fractions 1–4
Rf — retardation factor
SD — standard deviation
STZ — streptozotocin
TLC — thin-layer chromatography

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