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Antioxidant and antidiabetic properties of *Phyllanthus acidus* (L.) Skeels ethanolic seed extract

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Abstract

Oxidative stress is an important part of diabetic physiopathology; therefore, naturally derived antioxidants as therapeutic agents are needed. The present work examined the antioxidant and antidiabetic potentials of seed extract of *Phyllanthus acidus* (L.) Skeels. Ethanolic seed extract of *P. acidus* (PSE) was prepared using Soxhlet extraction. Preliminary phytochemical analysis, total phenolic (TPC), and total flavonoid (TFC) contents of PSE were determined. The *in vitro* inhibitory effects of PSE on 1,1-diphenyl-2 picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radicals, α-amylase, and α-glucosidase enzyme inhibitory activities were also assessed. Acute oral toxicity of PSE was carried out in female Sprague Dawley (SD) rats. PSE's hypoglycaemic effect was demonstrated in normoglycemic and streptozotocin-induced (STZ, 55 mg/kg, ip) diabetic rats. The TPC and TFC were found to be 3.19 mg of gallic acid equivalent/g (GAE/g), and 2.58 mg of quercetin equivalent/g of extract (QE/g), respectively. The inhibitory concentration 50% (IC_{50}) values, based on the inhibition of DPPH (IC_{50} 28.26 ± 0.39 µg/mL), ABTS (IC_{50} 23.44 ± 0.48 µg/mL) radicals, α-amylase (IC_{50} 26.83 ± 0.39 µg/mL), and α-glucosidase (IC_{50} 19.12 ± 0.26 µg/mL) enzymes were generally lower, which demonstrated their potent antioxidant and antidiabetic effects, respectively. Based on the acute oral toxicity test, two doses (100 and 200 mg/kg) of PSE were tested in normoglycemic rats. A significant ($p < 0.01$) fall in blood glucose was observed at 8 and 12 h after PSE (200 mg/kg) treatment. The same dose was tested in STZ rats and compared with glibenclamide (5 mg/kg). A significant ($p < 0.01$ and $p < 0.001$) decrease in the blood glucose level was observed after 8 and 12 h of PSE treatment, respectively. These findings suggest that PSE could be used as an active ingredient in herbal formulations to manage diabetes mellitus.

Introduction

Numerous recently discovered natural products are indispensable for both preventing and treating human diseases. Their efficacy makes them the treatment of choice over synthetic drugs. *Phyllanthus acidus* Skeels is a tree commonly known as the star gooseberry, and belongs to the family Euphorbiaceae. *Cicca acida* (Linn.) is a synonym of *P. acidus*, and used as an antibacterial (Sowmya *et al.,* 2018), hepatoprotective (Jain and Singhai 2011), anti-inflammatory (Hossen *et al.,* 2015), and antidiabetic agent (Ghosh Tarafdar *et al.,* 2016). *Phyllanthus* spp. are also considered as essential active ingredients in Unani and Ayurvedic systems of medicine (Krishnaveni and Mirunalini, 2010) due to their rich sources of phytochemicals that benefit human health (Huang *et al.,* 2017). Recent scientific studies have reported on the antioxidant (Hashim *et al.,* 2013, Nguyen *et al.,* 2017), α-amylase (Hashim *et al.,* 2013), α-glucosidase (Sulaiman and Ooi, 2014), cholinesterase (Moniruzzaman *et al.,* 2015) inhibitory, and antidiabetic (Nain *et al.,* 2012) effects of various *Phyllanthus* spp.

Traditionally, *P. acidus* fruits and leaves are used to treat ulcer, cough, scurvy, and asthma, and also used as an astringent and laxative. The extracts obtained from various parts of the *P. acidus* plant have cytotoxic (Vongvanich *et al.,* 2000), antihyperlipidemic (Chongsa *et al.,* 2014), and hypotensive (Leeya *et al.,* 2010) effects; they can also be used to treat cystic fibrosis (Sousa *et al.,* 2007). Traditionally, *P. acidus* fruits have been used for managing diabetes mellitus (DM) in many countries (Mao *et al.,* 2016). However, there is limited information on *P. acidus* seeds in managing hyperglycaemia, and further scientific studies are needed to support its use as an antidiabetic agent. The present work thus aimed to investigate the antioxidant, and carbohydrate-hydrolysing enzyme inhibitory effects of ethanolic extract of *P. acidus* seeds, and to evaluate its impact on blood glucose level in

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normoglycemic and streptozotocin-induced (STZ) diabetic rats.

Materials and methods

Reagents

1,1-diphenyl-2 picrylhydrazyl (200) was purchased from Merck (USA). 2,2-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid] (ABTS), acarbose, ascorbic acid (AA), gallic acid (GA), glibenclamide, quercetin, streptozotocin, α-amylase (source: *Aspergillus oryzae*), and α-glucosidase (source: *Saccharomyces cerevisiae*) were purchased from Sigma (USA). All other chemicals were of analytical grade.

Plant material

Fresh *P. acidus* fruits were harvested from local farms in Hyderabad, Telangana, India. The plant specimen containing the fruits was authenticated at the Department of Pharmacognosy, Kakathiya University, Warangal, India (accession number: KU/-FOP/1166). The seeds were separated from the fruit pulp, washed with water, and dried under sunlight for 1 - 2 d. The seeds were further dried in a hot air oven below 40°C to remove the excess moisture content before being coarsely powdered using a blender.

Extract preparation

The ethanolic extracts of *P. acidus* seeds were prepared using the Soxhlet extraction method (Chigurupati *et al.,* 2018). Briefly, 20 g of *P. acidus* seed powder was extracted with 250 mL of absolute ethanol. The powder was packed into the Soxhlet thimble, lined with a cotton pad until the Soxhlet siphon tube. The thimble was fitted with a condenser on its top, and with a 500 mL round-bottom flask (RBF) to its bottom. Extraction was initiated by heating (~75 - 80°C) the RBF using a heating mantle. This process was continued until the solvent in the Soxhlet siphon tube turned colourless. The solvent containing the seed extract was then cooled and concentrated using a rotary flash evaporator, and further freeze-dried.

Phytochemical analysis

Phytochemical analysis of ethanolic seed extract of *P. acidus* (PSE) was performed following standard methods (Chigurupati *et al.,* 2017) to identify the existence of phytoconstituents, such as alkaloids, flavonoids, phenols, mucilage, tannins, gums, glycosides, non-reducing sugars, saponins, proteins, monosaccharides, terpenoids, steroids, amino acids, carbohydrates, and reducing sugars.

Total phenolic content

The total phenolic content (TPC) in PSE was determined by the Folin-Ciocalteu method (Chigurupati *et al.,* 2019). PSE and GA were prepared at concentrations of 5, 10, 15, 20, and 25 µg/mL in 10 mL volumetric flasks. 100 µL of varying concentrations of PSE or GA, 100 µL of Folin-Ciocalteu reagent, and 2 mL of 2.5% Na_2CO_3 solution were added to the test tubes. The mixture was vortexed and incubated at room temperature away from light for 1 h. The absorbance was measured spectrophotometrically at 750 nm. All the experiments were performed in triplicate (n = 3). The quantifications of TPC in PSE were performed by a standard curve of GA, which acted as the standard phenolic compound, and was expressed as mg of GA equivalent/g of PSE (mg GAE/g)

Total flavonoid content

The total flavonoid content (TFC) in PSE was determined by the aluminium chloride ($AlCl_3$) colorimetric method (Chigurupati *et al.,* 2019). Similar concentrations (10, 20, 40, 60, 80, and 100 µg/mL) of PSE and quercetin were prepared in a 10 mL volumetric flask. The reaction mixture containing 1 mL of varying concentrations of PSE or quercetin, 0.7 mL of 5% $NaNO_2$, and 10 mL of ethanol (30% v/v) was vortexed and allowed to stand for 5 min. Then 0.7 mL of 10% $AlCl_3$ was added, and the mixture was allowed to stand for a further 6 min. Then, 10 mL of 1 mM/L NaOH was added, making a volume of 25 mL with aqueous 30% v/v ethanol, and allowed to stand for 10 min. The absorbance was measured spectrophotometrically at 450 nm. All the experiments were performed in triplicate (n = 3). The procedure was repeated for standard quercetin, and the calibration curve was plotted. The TFC in PSE was expressed as mg of quercetin equivalent/g of PSE (mg QE/g).

In vitro studies

DPPH radical scavenging assay

The DPPH radical scavenging activity of PSE was determined spectrophotometrically by adopting the earlier methods (Chigurupati *et al.,* 2016). The PSE and standard AA were prepared in various concentrations (10 – 1,000 µg/mL) using absolute alcohol. First, 0.5 mL of varying concentrations of PSE or AA were added to 0.5 mL of DPPH solution and incubated in the dark at room temperature for 20 min. The absorbance was measured spectrophotometrically at 517 nm. The percentage inhibition of DPPH free radical was calculated using Eq. 1. The inhibitory concentration 50% (IC_{50}) for PSE and AA to inhibit the DPPH radicals was calculated from the graph plot against percentage inhibition DPPH radical vs PSE or AA concentration.

% Inhibition = (absorbance $_{control}$ - absorbance PSE) / absorbance $_{control}$ × 100 (Eq.1)

ABTS radical scavenging assay

The ABTS free radical scavenging effect of PSE was determined following the procedures described by Chigurupati *et al.* (2016). An aqueous solution of $ABTS^+$ (7 mM/L) and potassium persulfate (2.45 mM/L) was added. The mixture was incubated for 12 - 16 h overnight in the dark at room temperature. Equal volumes (0.5 mL) of $ABTS^+$ solution and varying concentrations (10 – 1,000 µg/mL) of ethanolic solutions of PSE or AA were added, mixed, and subjected to incubation for 30 min. The absorbance was measured spectrophotometrically at 734 nm. The percentage of ABTS radical scavenging and IC_{50} values were calculated similarly to that of the DPPH method.

α-amylase and α-glucosidase inhibitory assay

PSE's α-amylase inhibitory effect was carried out following the methods described by Noreen *et al.* (2017). Briefly, to 0.5 mL of ethanolic solutions of PSE or acarbose (0.1 - 1 mg/mL), 500 µ L of 0.5 mg/mL α-amylase solution in 0.20 mM phosphate buffer (pH 6.9) was added. They were vortexed and incubated at 25°C for 10 min. A 1% starch solution in 0.02 M sodium phosphate buffer (500 µL) was added and further subjected to incubation for 10 min at 25°C. Then, 1 mL of dinitrosalicylate was added and boiled for 5 min, and cooled at room temperature. Then, 10 mL of distilled water was added, and the absorbance was measured spectrophotometrically at 540 nm.

PSE's α-glucosidase inhibitory effect was performed as described earlier by Hameed *et al.* (2019). The PSE was pre-incubated with 0.2 mL of α-glucosidase solution for 5 min, and 0.2 mL of sucrose was added to this reaction mixture, and incubated for a further 30 min at 37°C. The reaction was arrested by heating the reaction mixture to 90 – 100°C in a boiling water bath. The quantity of glucose liberated in the reaction mixture was determined by the GOD-POD method at 546 nm. The percentage inhibitory effect of the PSE and acarbose against α-amylase and α-glucosidase and their IC_{50} values were calculated.

In vivo studies

Experimental animals

Sprague Dawley (SD) rats of either gender with a mean body weight of 200 g were obtained from the animal house of Qassim University, Saudi Arabia, and acclimatised to laboratory environmental conditions. The rats were fed with standard rodent pellet food and purified drinking water *ad libitum.* Animals were maintained at an ambient room temperature of 25 ± 2°C and relative humidity (45 - 55%), and exposed to 12 h each of dark and light cycles. The Qassim University Human and Animal Ethics Committee approved 43 animals for the study protocol (QUHAEC07/CP/2018).

Acute oral toxicity study

Acute oral toxicity of PSE was carried out as per the OECD-423 test guideline (Kesavanarayanan *et al.,* 2013). A PSE (200 mg/mL) suspension was freshly prepared, using 0.5% carboxymethyl cellulose (CMC) solution, and orally fed to overnight fasted female SD rats at 2,000 mg/kg (3 animals/-dose/step; 10 mL/kg). The SD rats were frequently checked for clinical signs and mortality during the 24 h exposure period. After 24 h, animals were checked (at least once a day) for clinical signs and mortality for a total of 14 days.

Effect on normoglycemic animals

PSE's effect on normoglycemic animals was tested to understand their effect on blood glucose levels (BGL) at different periods after the single oral dose of PSE (Chackrewarthy *et al.,* 2010; Sharma *et al.,* 2014). The overnight fasted male rats were arbitrarily assigned to four groups (n = 5). The normal control (group 1) received 0.5% CMC solution (10 mL/kg). The positive control group (group 2) received glibenclamide (5 mg/kg). The test groups (groups 3 and 4) received PSE (100 and 200 mg/kg, respectively). Except for group 1, all the groups (2 – 4) received glucose (2 g/kg) (Yashwant Kumar *et al.,* 2011) 1 h before the respective treatment. The rats showing glucose level ≥ 7 mM/L were removed from the experiment. All treatments were made orally, using an oral gavage needle, and freshly prepared. The basal BGL (0 h) for all groups were measured using a one-touch glucometer from the blood sample collected from the tail vein under light ether anaesthesia. Blood samples were collected at 2, 4, 8, and 12 h after treatment, and their individual BGL and the percentage glycaemic changes were recorded.

Effect on diabetic animals

DM was induced in overnight fasted rats using single intraperitoneal injection (55 mg/kg) of freshly prepared STZ in ice-cold 0.01 M citrate buffer; pH adjusted to 4.5. After 6 h of STZ induction, the rats were given orally 5% w/v of glucose solution (2 mL/kg) for the next 24 h to protect them

from hypoglycaemic shock. After 48 h, the rats showing ≥ 12 mM/L of fasting blood glucose were considered diabetic, and selected for the experiment. The rats were randomly distributed into four groups (n = 5). The group 1 rats served as normal non-diabetic rats, and groups 2 - 4 were diabetic rats. Groups 1 and 2 were treated orally with 0.5 % CMC solution (10 mL/kg). Groups 3 and 4 received glibenclamide (5 mg/kg) and PSE (200 mg/kg), respectively. The tail vein BGL was estimated at 0, 2, 4, 8, and 12 h after drug administration using a glucometer (Chackrewarthy *et al.,* 2010; Sharma *et al.,* 2014).

Statistical analysis

The results obtained from *in vitro* studies were expressed as the mean ± standard deviation (SD), and all experiments were executed in triplicate. The IC_{50} values for *in vitro* antioxidant and antidiabetic studies were computed using the Graph Pad Prism Software (Version-5) using non-linear regression graph, which was plotted between the percentages of radical scavenging on the *x*-axis *versus* concentrations on the *y*-axis for antioxidant studies, and between the percentages of enzyme inhibition on *x*-axis *versus* concentration on the *y*-axis for antidiabetic studies. The results obtained from *in vivo* studies were expressed as mean ± standard error of mean (SEM). The statistical significance between the groups was tested using a one-way analysis of variance followed by Tukey's multiple comparison *post-hoc* test. A $p < 0.05$ (95% confidence intervals) were considered significant.

Results and discussion

Phyllanthus spp. are extensively used in the traditional system of medicine to treat numerous ailments, including DM, due to their rich diversity in phytochemicals (Nisar *et al.,* 2018). The Soxhlet extraction of *P. acidus* seeds with absolute ethanol produced a 55% extract yield. The phytochemical screening of PSE showed the presence of alkaloids, mucilage, steroids, flavonoids, phenols, terpenoids, and tannins. The qualitative phytochemical analysis, carried out on various parts of this plant, also indicated the presence of these active phytochemical constituents (Liliwirianis *et al.,* 2011; Nisar *et al.,* 2018).

P. acidus biological effect was reportedly associated with its high levels of phenolic and flavonoid (Jain and Singhai, 2011; Moniruzzaman *et al.,* 2015) contents, and the extracts possess strong antioxidant (Nguyen *et al.,* 2017) effect. The TFC in PSE was expressed in terms of QE (the standard curve equation: $y = 0.0002x + 0.0323$, $R^2 = 0.9789$) as shown in Figure 1(a) and possessed 2.26 ± 0.15 mg of QE/g of PSE. The TPC in PSE was expressed in terms of GAE (standard curve equation: $y = 0.0009x + 0.0779$, $R^2 = 0.9665$) as shown in Figure 1(b) and possessed 1.16 ± 0.2 mg of GAE/g of PSE.

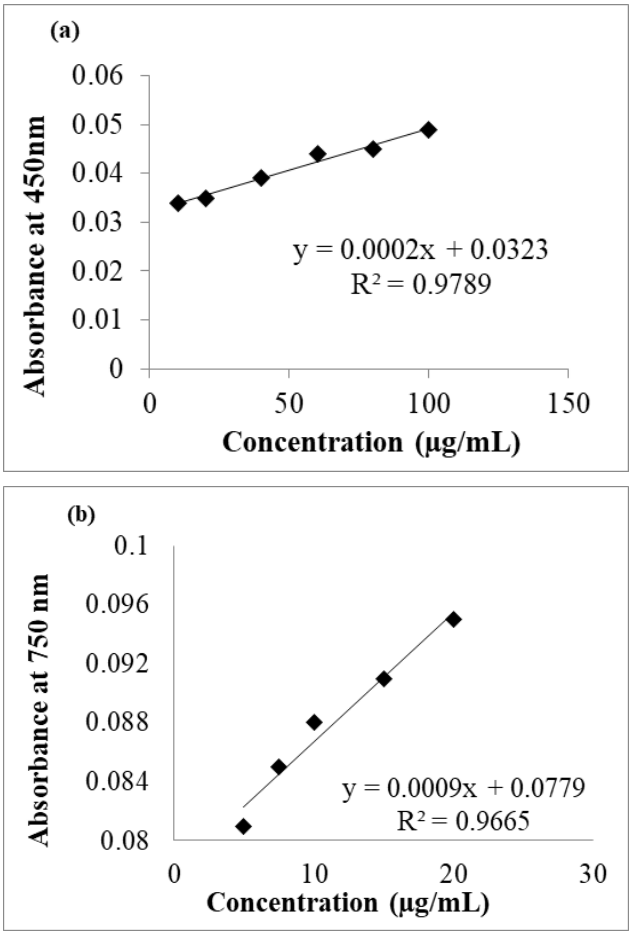

Figure 1. (a) The standard curve of quercetin to measure TFC; and (b) the standard curve of GA to measure TPC.

In vitro antioxidant activity was carried out on PSE using DPPH and ABTS methods, and measured for free radical scavenging. In the DPPH method, the reagent undergoes reduction by donating an electron or receiving a hydrogen atom, which changes colour from violet to colourless (Sahoo *et al.,* 2013). As for the ABTS method, a blue or green chromophore is produced due to the reaction between potassium persulfate and ABTS. Using the DPPH and ABTS methods, PSE was examined for *in vitro* antioxidant activity and measured for free radical scavenging. The PSE and AA (standard) were prepared at concentrations ranging from 10 – 1,000 µg/mL for both methods. As shown in Figure 2(a), PSE (IC_{50} 23.44 ± 0.48 µg/mL) exhibited good antioxidant activity AS compared to the standard drug, AA (IC_{50} 22.18 ± 0.74 µg/mL), in the ABTS method. The DPPH method, as shown in the graph in

Figure 2(b), supported the good antioxidant activity of PSE (IC_{50} 28.26 ± 0.39 µg/mL) AS compared to AA (IC_{50} 24.05 ± 0.39 µg/mL).

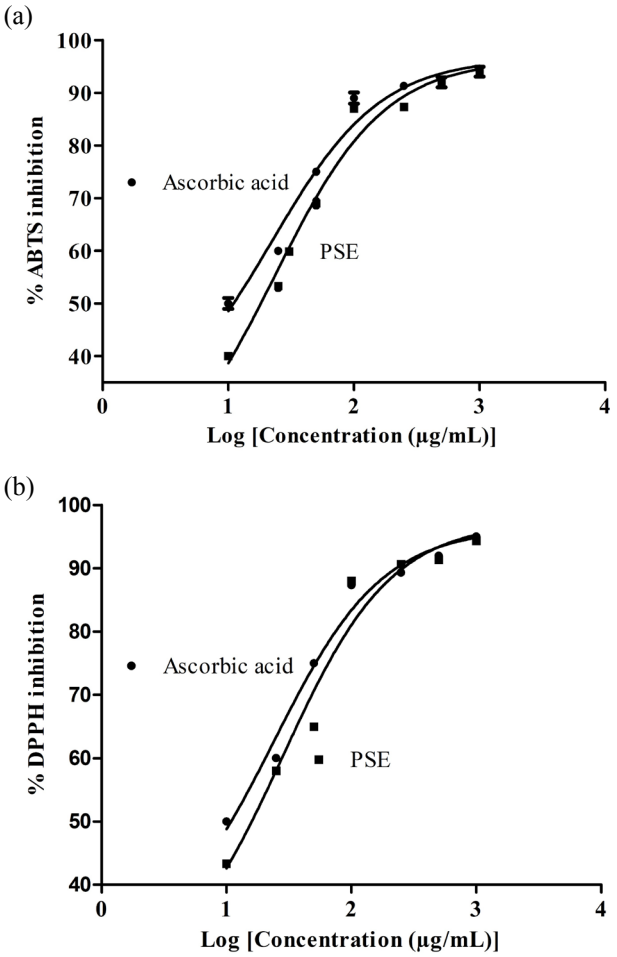

Figure 2. (a) ABTS radical, and (b) DPPH radical scavenging effects of PSE and AA (n = 3).

Plant-derived phytoconstituents influence glucose metabolism by reducing α-amylase and α-glucosidase activities (Alam *et al.,* 2019). The PSE extract and acarbose (standard) were used at concentrations 100, 200, 400, 800, and 1,000 µg/mL. In the α-amylase inhibition assay, acarbose revealed α-amylase inhibition activity with an IC_{50} value of 20.03 ± 0.47 µg/mL. Meanwhile, PSE showed an IC_{50} value of 26.83 ± 0.39 µg/mL, as shown in Figure 3. The PSE showed good inhibitory activity against α-amylase. In the α-glucosidase inhibition assay, the IC_{50} value of acarbose was found to be 17.31 ± 0.37 µg/mL, followed by PSE with an IC_{50} value of 19.12 ± 0.26 µg/mL, as shown in Figure 3. *P. acidus* α-amylase and α-glucosidase inhibitory activities might be attributed due to phenolic compounds and their antioxidant properties (Sulaiman and Ooi, 2014). In all the *in vitro* experiments, the square of correlation coefficients (R^2 value) showed the goodness of fit between the observed *versus* predicted values.

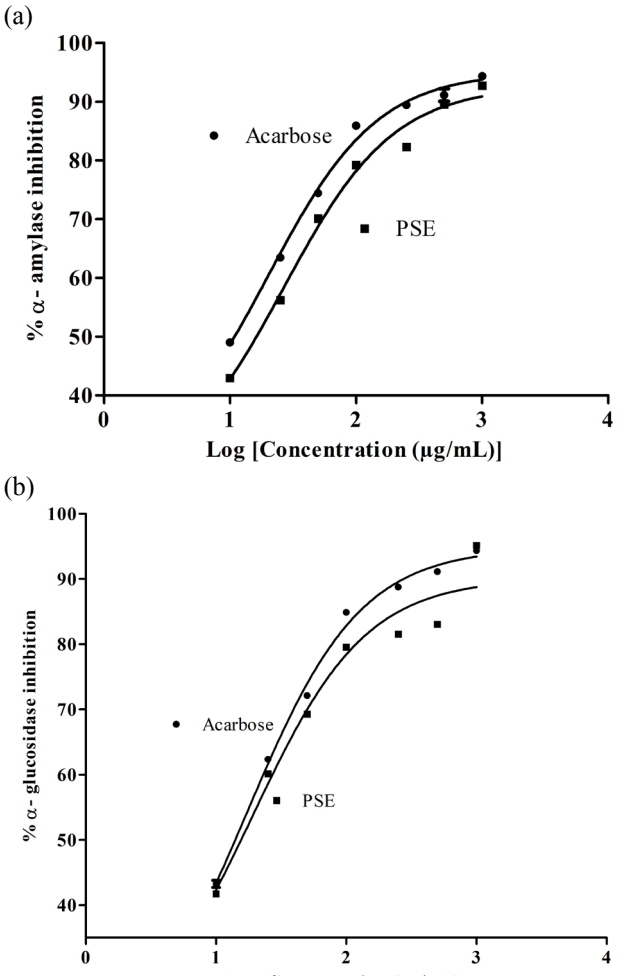

Figure 3. (a) α-amylase, and (b) α-glucosidase inhibitory effects of PSE and AA (n = 3).

The ethanolic leaf extract of *P. acidus* is orally safe at its highest oral dose (2,000 mg/kg) (Chaimum-aom *et al.,* 2017); hence, a limit test was conducted at a similar dosage with ethanolic seed extract. The acute toxicity test exhibited the normal behaviour of the treated animals. There was no mortality noticed at the high dose of 2,000 mg/kg. Thus, $1/10^{th}$ of the safer dose was selected as a therapeutic dose. Based on the acute toxic class method, the LD_{50} of PSE was found to be higher than 2,000 mg/kg.

As depicted in Table 1, normal rats treated with PSE (200 mg/kg) exhibited a significant post-dose decrease in BGL, $p < 0.01$ at 8 and 12 h, respectively, as compared to the control group. PSE-treated (100 mg/kg) rats showed a post-dose reduction in BGL, but it was found to be insignificant as compared to the control group. Moreover, standard drug treatment with glibenclamide in rats showed a significant post-dose decrease in BGL $p < 0.05$ at 4 h, and $p < 0.001$ at 8 and 12 h. The effect of PSE treatment on percent glycaemic change in normoglycemic and diabetic rats, at various time intervals of

Table 1. Effect of a single dose of PSE on BGL in normal rats.

Group	Blood glucose (mM/L)				
	0 h	2 h	4 h	8 h	12 h
Normal control	4.16 ± 0.29	4.18 ± 0.23 [↑0.48]	3.92 ± 1.88 [↓5.77]	4.51 ± 0.20 [↑8.41]	4.64 ± 0.14 [↑11.54]
PSE 100 mg/kg	4.52 ± 0.24	4.12 ± 0.26 [↓8.85]	3.57 ± 0.19 [↓21.02]	3.78 ± 0.18 [↓16.37]	3.80 ± 0.30 [↓15.93]
PSE 200 mg/kg	4.47 ± 0.42	4.25 ± 0.39 [↓4.92]	3.79 ± 0.27 [↓15.21]	3.27 ± 0.15[b] [↓26.85]	3.21 ± 0.14[b] [↓28.19]
Glibenclamide (5 mg/kg)	4.48 ± 0.2	3.90 ± 0.24 [↓12.95]	3.02 ± 0.35[a] [↓32.59]	2.88 ± 0.12[c] [↓35.71]	2.80 ± 0.08[c] [↓37.50]

Values are mean ± SEM; $n = 5$ in each group. [a] = $p < 0.05$; [b] = $p < 0.01$; [c] = $p < 0.001$, with respect to control. The values in parenthesis [ ] indicate the % increase (↑) or decrease (↓) in the BGL as compared to the BGL at 0 h. The % ↑ or ↓ in glycaemia was calculated using the formula $(G_0 - G_x / G_0) \times 100$, where, G_0 = initial BGL, and G_x = BGL at 2, 4, 8, and 12 h intervals (Algariri *et al.,* 2013).

the respective treatments, is shown in Tables 1 and 2, respectively. In normoglycemic rats, though PSE (100 mg/kg) treatment reduced BGL by 21.02%, a gradual decrease in the percentage of BGL (4.92 to 28.19%) was observed from 4 to 12 h of PSE (200 mg/kg) treatment respectively (Table 1). Hence, 200 mg/kg dose was selected and tested for efficacy in diabetic rats. Interestingly, the percent decrease in the BGL with PSE (200 mg/kg) and glibenclamide (5 mg/kg) treatment was similar at 8 and 12 h.

In normoglycemic rats, both PSE doses (100 and 200 mg/kg) reduced the BGL. However, PSE at the dose of 200 mg/kg showed significant ($p < 0.01$) blood glucose reduction at 8 h. Thus, PSE (200 mg/kg) was selected for the effect on BGL in diabetic induced rats. As depicted in Table 2, diabetes was induced by injecting STZ, and rats with BGL ≥ 12 mM/L were considered diabetic, and selected for further evaluation. Diabetic rats treated with PSE (200 mg/kg) exhibited a significant post-dose reduction in BGL $p < 0.01$ at 8 h, and $p < 0.001$ at 12 h, as compared to diabetic control rats. The standard drug treatment with glibenclamide in diabetic rats also showed a significant post-dose decrease in BGL $p < 0.01$ at 4 h, and $p < 0.001$ at 8 and 12 h. The blood glucose reduction with PSE treatment might be due to various phytochemicals that act, individually or synergistically, in glucose homeostasis by various mechanisms. Likewise, most constituents, including gallotannins, quercetin, and GA within *Phyllanthus* plant extracts, were tested with diabetic associated protein targets involved in glucose homeostasis (Nisar *et al.,* 2018; Srinivasan *et al.,* 2018).

Conclusion

The present work suggests that the *P. acidus* ethanolic seed extract could be an exciting source of

Table 2. Effect of a single dose of PSE on BGL in diabetic rats.

Group	Blood glucose (mM/L)				
	0 h	2 h	4 h	8 h	12 h
Control	4.32 ± 0.29	4.46 ± 0.37 [↑3.24]	4.02 ± 0.13 [↓6.94]	4.08 ± 0.12 [↓5.56]	4.12 ± 0.19 [↓4.63]
Diabetic control	23.80 ± 2.50	20.98 ± 2.75 [↓11.85]	20.38 ± 1.96 [↓14.37]	19.46 ± 1.31 [↓18.24]	18.44 ± 1.05 [↓22.52]
PSE 200 mg/kg	25.82 ± 1.10	22.68 ± 1.78 [↓12.16]	19.88 ± 0.81 [↓23.01]	13.00 ± 1.36[b] [↓49.65]	11.8 ± 0.39[c] [↓54.30]
Glibenclamide (5 mg/kg)	24.38 ± 1.41	15.96 ± 0.86 [↓34.54]	14.44 ± 0.76[b] [↓40.77]	10.58 ± 0.94[c] [↓56.60]	8.52 ± 0.76[c] [↓65.05]

Values are mean ± SEM; $n = 5$ in each group. [a] = $p < 0.05$; [b] = $p < 0.01$; [c] = $p < 0.001$, with respect to diabetic control. The values in parenthesis [ ] indicate the % increase (↑) or decrease (↓) in the BGL as compared to the BGL at 0 h. The % ↑ or ↓ in glycaemia was calculated using the formula as shown in Table 1.

flavonoids and phenolic compounds with promising antioxidant and carbohydrate hydrolysing enzyme inhibitory effects. A high dose of this extract is orally safe and exhibits *in vivo* antidiabetic activity. The ethanolic extract of the seeds of *P. acidus* could be a potential source of nutraceuticals. It could be used as an alternative therapeutic approach to managing postprandial hyperglycaemia and associated oxidative stress.

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