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FULL ARTICLE

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Evaluation of the in vitro α-amylase inhibitory, antiglycation, and antioxidant properties of *Punica granatum* L. (pomegranate) fruit peel acetone extract and its effect on glucose uptake and oxidative stress in hepatocytes

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Abstract

Punica granatum L. (pomegranate) is a widely eaten fruit. The antidiabetic, antioxidative, and antilipidemic properties of the hydroalcoholic extracts of the different plant's parts have been extensively studied, with scarce information on the acetone extract (ACE). This study investigated antidiabetic, antioxidative, and antiobesogenic properties of ACE of the fruit peel. Preliminary data showed that ACE showed stronger antioxidant (radical-scavenging IC_{50} = 1.56 μg/ml) and α-amylase inhibitory (IC_{50} = 10.6 μg/ml) properties than the hydroalcoholic extracts and Acarbose. The ACE inhibited protein glycation and lipase activity. In hepatocytes, ACE impaired oxidative stress-induced lipid peroxidation and reduced glutathione depletion but increased glucose uptake without decreasing the cell viability. HPLC analysis showed predominant presence of bioactive phenolic acids (ferulic, caffeic, and gallic acids) in this extract. This study suggests that ACE of *P. granatum* fruit peel may be an understudied extract that contains potent antidiabetic and antioxidative bioactive principles with minimal toxicity.

Practical applications

Plant derived medicines have been an affordable and effective alternative therapy for many metabolic diseases, including diabetes. The fruit juice and fruits of pomegranate is widely consumed for the palatable taste and cardiovascular benefits. This study provides preliminary experimental evidences confirming that of the acetone extracts of pomegranate fruit peel, which has been sparsely studied, may possess more potent antidiabetic and antioxidative property than to the routinely studied hydroalcoholic counterparts. Additionally, the acetone extract is rich in bioactive phenolic acids, suggesting that the acetone extract of pomegranate fruit peel may be a promising candidate for further antidiabetic study and a source of bioactive principles for the management of diabetes and oxidative complications.

KEYWORDS

acetone extract, antidiabetic, antioxidative, fruit peel, high performance liquid chromatography (HPLC), pomegranate, *Punica granatum* L.

Abbreviations: AAE, ascorbic acid equivalent; ACE, acetone extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, effective concentration (μg/ml) required to cause 50% of effect; ETE, ethanol extract; FRAP, ferric ion reducing antioxidant power; GAE, gallic acid equivalent; GSH, reduced glutathione; HPLC, high performance liquid chromatography; IC_{50}, inhibition concentration (μg/ml) required to cause 50% inhibition; QE, quercetin equivalent; ROS, reactive oxygen species; T2D, type 2 diabetes; TE, Trolox equivalent; WTE, water extract.

1 | INTRODUCTION

The global prevalence of diabetes has been predicted to increase drastically in the coming decades, which will pose socioeconomic burden on many countries (IDF, 2017), particularly, the less developed African countries, where western drugs are relatively less affordable. Diabetes mellitus is a group of metabolic disorders characterized by a state of chronic hyperglycemia resulting from insufficient insulin secretion or action (Farzaei, Morovati, Farjadmand, & Farzaei, 2017). Diabetes has two major types, namely type 1 and type 2 (T2D) diabetes. Type 1 diabetes is attributed to lack of insulin secretion, while T2D is characterized by reduced sensitivity of target cells to insulin (Farzaei et al., 2017).

Type 2 diabetes is the most prevalent of the types of diabetes accounting for about 90% of cases (Oyebode et al., 2018). It is linked to both inadequate insulin release and deficient action on targeted organs (Chukwuma, Mopuri, Nagiah, Chuturgoon, & Islam, 2018). In T2D, insulin secretion does not adequately maintain glucose homeostasis in the face of increasing peripheral insulin resistance, which may gradually and detrimentally affect the mass and function of the pancreatic β-cell. Those with T2D are often obese. Obesity and poor adipocyte differentiation contribute to insulin resistance, which causes dyslipidemia in T2D (Goossens, 2008). In fact, obesity is among the major risk factors of diabetes, which include unhealthy dieting, sedentary lifestyle and peripheral insulin resistance.

Diabetes is associated with several microvascular and macrovascular complications (Oyebode et al., 2018). Oxidative stress is a major mediator or culprit in the development and progression of diabetic complications, due to the increase of prooxidants during diabetes. Prooxidants, such as reactive oxygen species (ROS) and free radicals oxidatively damage biological molecules, cells, tissues, and organs, thus, leading to several diabetic complications (Pourghassem-Gargari, Abedini, Babaei, Aliasgarzadeh, & Pourabdollahi, 2011).

In spite of the available Western drugs for diabetes, plant-derived medicine remains a popular alternative and complementary therapy for diabetes and related diseases, especially in developing and low-income African countries, where Western drugs are difficult to afford. In fact, a massive portion of the African population still rely on folk medicine to manage their health. This is largely due to the presence of antidiabetic and antioxidative phytochemicals, like polyphenols, in many indigenous plants (Upadhyay & Dixit, 2015).

Punica granatum L. is a fruit-bearing plant, commonly known as pomegranate and belonging to the family Lythraceae. It is grown in America, Northern India, Iran, and throughout the Middle East region (Huang et al., 2005). The fruit is commonly consumed as food and fruit juice and wine are widely consumed as refreshing beverages with cardiovascular benefits (Aviram et al., 2002). Traditionally, the flowers have been used to treat diabetes in Indian Unani and Ayurvedic medicines (Aviram et al., 2002). Several studies have reported the antidiabetic, antioxidative, and antiobesogenic properties of the flowers, leaves, fruit and fruit peel, seeds, seed oil, and juice of the plant, which have been attributed to the ellagic polyphenol contained in the different plant parts (Sun, Zhou, & Wang, 2019). The edible part of the fruit is rich in ellagitannins, and anthocyanins, while the fruit peel contains condensed tannins, catechins, gallocatechins, and prodelphinidins (Gómez et al., 2013; Nasr, Ayed, & Metche, 1996; Plumb, De Pascual-Teresa, Santos-Buelga, Rivas-Gonzalo, & Williamson, 2002).

Huang et al. (2005) reported gallic acid as a major component of the methanolic extract of the flowers, which may be partly responsible for its anti-hyperglycemic, as well as glucose transporter type 4 expression and insulin sensitivity modulatory effects of the extract in Zucker diabetic fatty rats. Other studies further confirmed that the ethanolic extract of the flower ameliorated diabetes-induced hyperglycemia, insulin insensitivity, dyslipidemia and oxidative stress in STZ-induced (Manoharan et al., 2009) and high-fat-diet-induced (Tang et al., 2018) diabetic rats. In addition, polyphenol rich (HPLC: 8.3% of ellagic acid, 1.49% of gallic acid, 0.79% of pyrogallic acid, and 18.27% of tannic acid) (Yu et al., 2017) and hydroalcoholic extracts (Adnyana, Sukandar, Yuniarto, & Finna, 2014) of the leaves showed antiobesogenic activities by inhibiting pancreatic lipase activities and reducing fat intestinal absorption.

Furthermore, the fruit and its components have be extensive studied and shown to have pharmacological credence. Extracts (water, methanol, and ethanol) or juice of the fruit components (edible parts, seeds, arils, and peel) reduced blood glucose, increased liver glycogen content and insulin secretion, improved glucose tolerance, modulated expression of insulin signaling factors in diabetic rats, improved lipid profile, reduced lipid peroxidation and hepatic damage and modulated the antioxidant status of diabetic and oxidative stress-induced rats (Chidambara Murthy, Jayaprakasha, & Singh, 2002; Das et al., 2001; Das & Sama, 2009; Gharib & Kouhsari, 2019; Prasetyastuti, Anthony, Rahman, Ngadikun, & Sunarti, 2014; Salwe, Sachdev, Bahurupi, & Kumarappan, 2015; Thanh, Huyen, Van Khanh, Thu, & Tung, 2019). In high-fat-diet-induced obese rats, the fruit juice reduced blood glucose and weight gain, improved satiety and insulin secretion and modulated the mRNA expression of several lipid and glucose metabolizing enzymes (Ahmed, Samir, El-Shehawi, & Alkafafy, 2015).

In vitro, the hydro-ethanolic and methanolic extracts or isolates of the fruit, its peel and/or seeds showed radical-scavenging and ferric reducing antioxidant properties (Hasan, Redha, & Mandeel, 2018; Middha, Usha, & Pande, 2013; Singh, Chidambara Murthy, & Jayaprakasha, 2002; Zeghad, Ahmed, Belkhiri, Vander Heyden, & Demeyer, 2019), as well as α-amylase (Hasan et al., 2018; Jain, Viswanatha, Manohar, & Shivaprasad, 2012) and lipase inhibitory activities (Shamsiya, Manjunatha, & Manonmani, 2016). In the methanol extract of the fruit peel, qualitative analysis showed the presence phenols, tannins, flavonoids, coumarins, quinones, steroids, triterpenoids, proanthocyanidins, and reducing sugars (Hasan et al., 2018; Middha et al., 2013), while HPLC analysis confirmed the presence of quercetin, rutin, gallic acid, ellagic acid, and punicalagin (Khalil, Khan, Shabbir, & Rahman, 2017; Middha et al., 2013). Ellagic acid, gallagic acid, punicalagin, and derivatives, among others were identified in the acetone extract of the fruit peel using mass spectroscopic technique (Abid et al., 2017), while phenolic acids and flavonoids,

including catechin, caffeic acid, epicatechin gallate, cinnamic acid, and quercetin were identified in methanol extract of the edible fruit using HPLC analysis (Zeghad et al., 2019).

From the above, most pharmacological studies have focused on the hydroalcoholic or methanolic extracts of the different parts of the plants, while the fruit peel acetone extract, which has been previously shown to have lipase inhibitory activity (Lee, Chen, Liang, & Wang, 2017) remain understudied. In fact, preliminary data of the present study showed that the phenol and flavonoid contents, as well as the in vitro antioxidant and α-amylase inhibitory activities of the fruit peel acetone extract outperformed its water and ethanol counterparts and acarbose. Therefore, the present study was done to investigate the antidiabetic, antioxidative, and antilipidemic effects of the fruit peel acetone extract of *P. granatum*.

2 | MATERIALS AND METHODS

2.1 | Plant sample and extract preparation

Fresh and healthy "Wonderful" pomegranate fruits were obtained from the Postharvest research laboratory of Stellenbosch University, South Africa. The fruits were peeled manually using a knife. The arils were removed from the fruit, and the peels were collected in a bowl. The peels oven-dried at 60°C (Model No. 072160, Prolab instrument, Sep Sci., South Africa). The resulting oven-dried peels were pulverized using a milling machine, sieved (0.15-mm sieve), and stored at 4°C.

Fifty grams (50 g) of plant material was separately extracted in 500 ml of water (WTE), ethanol (ETE), and acetone (ACE) by agitating with an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 hr. Thereafter, the extracts were filtered (Whatmann No. 1 filter paper). ETE and ACE were concentrated under vacuum to dryness using a rotatory evaporator (Strike 202 Steroglass, Italy), while WTE was recovered using a Xeretec freeze dryer (Ceramic Filter Core Drier, Model: CD 052). The dried extracts were stored in clean vials at 2–8°C. Stock concentrations of extract were freshly prepared for assays by dissolving in water (for WTE) or 10% of DMSO (for ETE and ACE).

2.2 | Measurement of total phenol and flavonoid

The method reported by Chukwuma, Islam, and Amonsou (2018) was used to measure the total phenol content in extracts at a concentration of 60 μg/ml. Briefly, 25 μl of extracts (60 μg/ml final concentration in reaction volume) or gallic acid standard (3.75–120 μg/ml final concentration in reaction volume) was incubated with 125 μl of 10 times diluted Folin–Ciocalteu reagent (Merck, South Africa) and 100 μl of 0.7 M Na_2CO_3 for 30 min at room temperature in a 96-well plate. Thereafter, absorbance was measured at 765 nm (SpectraMax M2 microplate reader, Molecular Devices, California, USA). Gallic acid standard curve was used to compute the phenol content and expressed as mg/g GAE using the following formula:

$$\text{Phenol content}\left(\text{mg/g GAE}\right) = \frac{C \times SV}{M}$$

where, "*C*" is the concentration (μg/ml) extrapolated from gallic acid standard curve; "*SV*" is the sample or extract volume (ml), and "*M*" is the mass (g) of the sample in *SV* (ml) of the sample solution; "GAE" means "gallic acid equivalent".

For flavonoid content, the method reported by Lee, Shukla, Kim, and Kim (2015) was used with some modifications. Extracts were tested at 60 μg/ml. The reaction mixture containing 20 μl extracts (60 μg/ml final concentration in reaction volume) or quercetin standard (3.75–120 μg/ml final concentration in reaction volume), 80 μl absolute ethanol and 100 μl of 2% of $AlCl_3$ was incubated for 1 hr at 25°C, before absorbance was measured at 430 nm. A quercetin standard curve was used to compute the flavonoid content of extracts and expressed as mg/g QE using the following formula:

$$\text{Flavonoid content}\left(\text{mg/g QE}\right) = \frac{C \times SV}{M}$$

where, "*C*" is the concentration (μg/ml) extrapolated from quercetin standard curve; "*SV*" is the sample or extract volume (ml), and "*M*" is the mass (g) of the sample in *SV* (ml) of the sample solution; "QE" means "quercetin equivalent".

2.3 | High-performance liquid chromatography

HPLC-diode array detection analysis was performed on the acetone extract (ACE) using an Agilent 1100 series (Agilent, Waldbronn, Germany) instrument equipped with photo diode array, autosampler, column thermostat and degasser. A Phenomenex: Luna 5 μm C_{18} (2) (150 × 4.6 mm; 5 μm particle size) column was used as the stationary phase. Water containing 0.1% of formic acid (A) and acetonitrile (B) served as mobile phases at a flow rate of 1 ml/min. Gradient elution was applied as follows: Initial ratio 95% A: 5% B, keeping for 10 min, changed to 90% A: 10% B in 10 min, changed to 70% A: 30% B in 10 min, to 50% A: 50% B in 10 min, maintaining for 0.5 min and back to initial ratio in 0.5 min. Temperature was set at 30°C. Extract or standards were dissolved in HPLC grade methanol (2 mg/ml) and the injection volume was 20.0 μl. Chromatograms were recorded at 254 nm.

2.4 | 2,2-diphenyl-1-picrylhydrazyl (DPPH) and scavenging activity

This was measured using a previous method (Sanni et al., 2019). Different concentrations (7.5–60 μg/ml in reaction mixture) of extracts or standards (ascorbic acid and Trolox) or their solvents (control) were mixed with 37.5 μl of a 0.3 mM of DPPH solution to make a final volume of 112.5 μl. Absorbance was measured at

517 nm after a 30 min incubation in the dark and the radical-scavenging activity (%) was computed relative to the control using the following formula:

$$\text{Scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.5 | Nitric oxide radical-scavenging activity

The method reported by Oyedemi, Bradley, and Afolayan (2010) was used for this assay. Equal volumes (75 µl) of extract or standards (ascorbic acid and Trolox) at increasing concentrations (7.5–60 µg/ml in reaction mixture) or their solvents (control) and sodium nitroprusside (10 mM in 50 mM of phosphate buffer, pH 7.4) was mixed and incubated for 2 hr at 37°C. Thereafter, an equal volume of Greiss reagent (Merck, South Africa) was used to estimate NO_2^- formation at 546 nm. Scavenging activity of samples was computed as the inhibition of NO_2^- formation using the following formula.

$$\text{Scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.6 | Fe^{3+} reducing antioxidant power (FRAP)

This was measured using previous a method (Sanni et al., 2019). The reaction mixture containing 25 µl each of extracts (at 60 µg/ml in reaction mixture) or gallic acid, ascorbic acid and Trolox standards (3.75–120 µg/ml in reaction mixture), 0.2 M of phosphate buffer (pH 6.6), and 1% of potassium ferricyanide was incubated for 20 min at 50°C, before acidifying with 25 µl of 10% TCA. Thereafter, 100 µl of distilled water and 50 µl of 0.1% $FeCl_3$ solution were added, successively, and absorbance was measured at 700 nm. The FRAP of the extracts was determined from gallic acid, ascorbic acid, and Trolox standard curves and computed as mg/g equivalent of the standards using the following formula:

$$\text{FRAP}\ (\text{mg/g GE or AAE or TE}) = \frac{C \times SV}{M}$$

where, "*C*" is the concentration (µg/ml) extrapolated from gallic acid (for "GE") or ascorbic acid (for "AAE") or Trolox (for "TE") standard curves; "*SV*" is the sample or extract volume (ml), and "M" is the mass (g) of the sample in *SV* (ml) of the sample solution; "GAE," "AAE," and "TE" mean "gallic acid equivalent," "ascorbic acid equivalent," and "Trolox equivalent," respectively.

2.7 | α-amylase inhibition

Enzyme inhibition assays were performed using the method of Sanni et al. (2019). All three extracts were preliminarily tested at a single concentration of 35 µg/ml. The α-amylase inhibition of acetone extract (ACE) outperformed the WTE and ETE, and thus, was further studied for dose response (7.5–60 µg/ml). To perform this assay, 75 µl of different concentrations (7.5–60 µg/ml) of the extracts or positive control (acarbose) or their solvents (control) was mixed with 75 µl of 3 U/ml porcine pancreatic amylase (Sigma Aldrich, South Africa) solution (dissolved in100mM phosphate buffer, pH 6.8) in a 2 ml vial and incubated at 37°C for 15 min. A 75 µl of 1% of starch (dissolved in100mM phosphate buffer, pH 6.8) was then added and the mixture was incubated at 37°C for 30 min. Thereafter, 75 µl of dinitrosalicylate (DNS) color reagent was added before allowing the mixture to boil for 10 min. After cooling, each vial was centrifuged (5 min at 5,000 rpm) and a 200 µl aliquot of supernatant was transferred into a 96-well plate and absorbance was then read at 540 nm. The enzyme inhibition (%) of the samples was computed using the following formula:

$$\text{Enzyme inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.8 | Pancreatic lipase inhibition

The pancreatic lipase inhibitory activity of the ACE was measured using a previous method (Oliveira et al., 2015) with some modification. Briefly, porcine pancreatic lipase extract (Sigma Aldrich, South Africa) was homogenized (2 mg/ml) in ice-cold 100 mM of Tris-HCl buffer (pH 8.2) and the enzyme solution (supernatant) was recovered at 2,000× *g* for 5 min at 4°C. A 10 µl of increasing concentrations of the extract (7.5–60 µg/ml in reaction mixture) or Orlistat (0.75–6 µg/ml in reaction mixture) or the solvent (control) was mixed with 10 µl of the enzyme solution in a 2 ml vial and incubated for 10 min at 37°C. Thereafter, 480 µl of pre-warmed (37°C) substrate (p-Nitrophenyl-palmitate) solution dissolved in isopropanol and buffer was added and incubated for 30 min at 37°C. Concentrations of substrate and isopropyl alcohol in final reaction volume were 530 µM and 25% v/v, respectively. After incubation, the enzyme activity was stopped by immersing vials in boiling water for 10 min. Thereafter, the absorbance of a 200 µl aliquot of the supernatant (2,500× *g* for 10 min) was measured in a 96-well plate at 410 nm. The enzyme inhibition (%) was computed using the "Equation 2" formula.

$$\text{Enzyme inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.9 | Glycation inhibition

The protein glycation inhibition of the ACE was measured at 7.5–60 µg/ml using a previous method (Nakagawa, Yokozawa, Terasawa, Shu, & Juneja, 2002) with some modifications. A 50 µl volume of different concentrations (7.5–60 µg/ml in reaction mixture) of the extract or standard (aminoguanidine) or their solvents (control) was incubated with 50 µl each of glucose (90 mg/ml) and bovine serum albumin (10 mg/ml) in the dark for 3 weeks at 37°C using black 96-well plates. Thereafter, fluorescence was measured at

Ex/Em = 360 nm/420 nm, and the glycation inhibition was computed using the following formula:

$$\text{Glycation inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.10 | Measurement of protective effects against induced oxidative damage in Chang liver cells

This assay was modified from a previous method (Sanni et al., 2019). Human Chang liver cells (ATCC® CCL-13™, American Type Culture Collection, Virginia, USA) were cultured with (Eagle's minimum essential medium supplemented with 10% of heat-inactivated fetal bovine serum) in a humidified CO_2 incubator (NÜVE EC 160, Ankara, Turkey) set at 5% of CO2, 95% of oxygen, and 37°C. At about 90% confluence, cells were seeded in 200 µl medium in a 48-well plate at a density of 10,000 cells per well and allowed to attach to plate for 36 hr in the CO_2 incubator. Thereafter, used medium was replaced with 200 µl of fresh culture medium containing different concentrations (15 to 120 µg/ml in final assay volume) of ACE or standards (ascorbic acid and gallic acid) and incubated for 30 min. The control and negative control contained equivalent volume of fresh media (contained equivalent volume of vehicle for extract or standards). After incubation, 50 µl of $FeSO_4$ (7 mM in final 250 µl assay volume) was added to the test and negative control to induce oxidative stress, while equal volume of water was added to the control. After 30 min incubation, 250 µl of cold Phosphate Buffer Saline containing Triton X 100 (1% v/v in final volume) was added to lyse cells for 1 hr on ice with agitation, before aspirating each well, vortexing and centrifuging (10,000 g for 10 min at 4°C) to obtain the supernatant. The level of lipid peroxidation and reduced glutathione (GSH) was measured in the supernatant of the cell lysate (Mchunu et al., 2019; Sanni et al., 2019). Experiment was conducted in three replicates of two biological repeats.

To measure lipid peroxidation, assay mixture containing 8.1% of sodium dodecylsulfate (SDS) (40 µl), 20% of acetic acid (pH 3.5) (150 µl), 0.25% of thiobarbituric acid (400 µl), distilled water (170 µl), and 40 µl of sample (supernatant) or malondialdehyde (MDA) standard series (0, 7.5, 15, 22.5, and 30 µM in 800 µl final volume of assay mixture) was heated at 95°C for 30 min in a sand bath. After cooling, absorbance was measured at 532 nm. Lipid peroxidation was expressed as MDA equivalent by extrapolating for MDA standard curve.

To measure GSH concentration, equal volumes (150 µl) of 10% of TCA and sample were first mixed to precipitate the proteins in samples. Supernatants (centrifuged at 2,000 rpm for 10 min) of precipitated sample (or GSH standard at 0.02–200 µM in reaction volume), 500 µM of 5,5′-dithio-bis-[2-nitrobenzoic acid (DTNB) and sodium phosphate buffer (200 mM, pH 7.8) were mixed in a volume ratio of 2 (30 µl):1 (15 µl):6 (180 µl), respectively. Absorbance of the mixture was measured at 415 nm after 15 min incubation at room temperature. The plot of GSH standard concentrations was used to extrapolate the GSH concentration of samples.

Percentage inhibition of lipid peroxidation and GSH depletion was computed using the following formula:

$$\text{Lipid peroxidation inhibition}(\%) = \frac{\text{TBARS of negative control} - \text{TBARS of test}}{\text{Absorbance of negative control}} \times 100$$

$$\text{GSH depletion inhibition}(\%) = \frac{(GSH_{Con} - GSH_{Neg}) - (GSH_{Con} - GSH_{Test})}{(GSH_{Con} - GSH_{Con})} \times 100$$

where, GSH_{Con}, GSH_{Neg}, and GSH_{Test} are the concentrations of GSH in the control, negative control, and test (ACE or standards) groups, respectively.

The percentage inhibitions were used to compute the IC_{50} values.

2.11 | Measurement of glucose utilization study in Chang liver cells

Assays was performed according to previously reported methods (Oyedemi, Koekemoer, Bradley, van de Venter, & Afolayan, 2013; van Huyssteen, Milne, Campbell, & van de Venter, 2011), with some modifications. Chang liver cells were seeded at a density of 6,000 cells/well in 96-plate well at volumes of 200 µl/well of growth medium (EMEM supplemented with 10% of heat-inactivated fetal bovine serum) and incubated in a CO_2 incubator at 5% of CO_2, 95% of oxygen and 37°C. After about 72 hr, medium was replaced with 200 µl of fresh RPMI-1640 glucose-free medium containing either DSMO (not more than 0.5% in final volume; control), metformin (1 µM in final volume) or extract (12.5, 25 and 50 µg/ml in final volume), as well as 8 mM of glucose and 0.1% of bovine serum albumin. After three hours of incubation at 37°C, 20 µl aliquot from each well was used to measure glucose concentration (µM) using the Glucose (GO) Assay Kit (Sigma Aldrich, South Africa) according to the kit protocol manual. Assay was performed in five replicates of two biological repeats. Glucose uptake was computed relative to the control using the following formula:

$$\text{Glucose uptake } (\%) = \frac{\Delta GC \text{ of test} - \Delta GC \text{ of control}}{\Delta GC \text{ of test}} \times 100$$

where "ΔGC" means change in glucose concentration (i.e., initial—final glucose concentration in incubation solutions).

2.12 | Cell viability inhibition assay

The effect of the ACE on cell growth or viability was done on Chang liver and Vero kidney cell lines using standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Chang liver and Vero kidney (ATCC® CCL-81™, American Type Culture Collection, Virginia, USA) cell lines were seeded in 100 µl medium in 96-well plates at a concentration of 1 × 105 cells/ml and incubated for 24 hr humidified CO2 incubator (NÜVE EC 160, Ankara, Turkey) set at 5% of CO_2,

95% of oxygen and 37°C to allow cells to attach to the bottom of the wells. Thereafter, used medium was replaced with 100 µl of fresh culture medium containing different concentrations (0.1 to 100 µg/ml in final assay volume) of ACE and not more than 0.5% of DMSO. Controls included cells exposed to 0.5% of DMSO. After the 36 hr treatment period, 100 µl (0.5 mg/ml in final volume) of MTT reagent (Sigma Aldrich, South Africa) was added to wells, the plate was swirled gently to mix content and incubation continued for 3 hr. Thereafter, wells were aspirated, and cells were washed with 100 µl of phosphate-buffered saline (PBS). A 100 µl of MTT de-staining or solubilization solution was added and absorbance was measured using a plate reader (Multiskan Go, Thermofisher Scientific) at 570 nm wavelength. Media-sample blank was included and utilized to normalize the results. The samples were evaluated in two independent biological repeats and each sample was evaluated in triplicate for each biological repeat. The cell growth/viability inhibition was computed using the following formula:

$$\text{Cell growth inhibition}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.13 | Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple range post hoc tests (IBM, SPSS, version 23) and *p* values less than .05 were considered significant when comparing groups. IC_{50} and EC_{50} values were computed using the GraphPad Prism 8 software.

3 | RESULTS AND DISCUSSION

The fruit and the fruit juice of *P. granatum* is widely consumed due to the palatable taste and cardiovascular medicinal properties (Aviram et al., 2002). The hydroalcoholic and methanolic extracts of the different parts of *P. granatum*, particularly the fruit parts (seed and peel), as well as the fruit juice have been extensively studied as potent antioxidant and antidiabetic agents, while there is little or no experimental data on the acetone extract. Data of this study showed that the acetone extracts outperformed the aqueous and ethanol extracts of the fruit peel and contained more amounts of polyphenols and flavonoids, suggesting that it may be a neglected extract with bioactive principle for diabetes and related disorders.

3.1 | Yield and phytochemical characterization of extracts

The yield of the WTE, ETE, and ACE were 23.8, 12.5, and 10.4%, respectively. The polyphenol and flavonoid contents of ACE (338 ± 20 m/g GAE and 60.8 ± 9.3 mg/g QE, respectively) were significantly ($p < .05$) higher than that of WTE and ETE (Table 1). Also, the polyphenol and flavonoid contents of ACE were higher than those reported for methanol, ethanol, and ethyl acetate extracts of the fruit peels of different Pakistani varieties ("Desi," "Kandhari," and "Badana") of pomegranate (Khalil et al., 2017; Khalil, Khan, Shabbir, & Rahman, 2018). Consistent trend of polyphenol and flavonoid content has also been reported by several other studies when comparing the fruit peel acetone extracts to other extracts (Abid et al., 2017; Thitipramote et al., 2019; Yasoubi, Barzegar, Sahari, & Azizi, 2007). Contrarily, Nuamsetti, Dechayuenyong, and Tantipaibulvut (2012) reported a lower phenol content in the acetone extract of the fruit's peel compared to the ethanol and hot water extracts. These discrepancies may be attributed to the different breeds of pomegranate fruit (South African vs. Thia grown) and extraction methods (e.g., hot vs. normal water extraction), among other factors.

TABLE 1 Preliminary in vitro phytochemical, antioxidant, and antidiabetic data comparing the WTE, ETE, and ACE of *P. granatum* fruit peel

Parameter or in vitro analysis	WTE	ETE	ACE	Acarbose	Ascorbic acid	Trolox
Total phenol content (mg/g GAE)	208 ± 15[a]	198 ± 6[a]	338 ± 20[b]	ND	ND	ND
Total flavonoid content (mg/g QE)	60.8 ± 9.3[a]	52.3 ± 11.1[a]	79.5 ± 4.1[b]	ND	ND	ND
FRAP						
(mg/g GAE)	158 ± 6[b]	138 ± 11[a]	227 ± 12[c]	ND	ND	ND
(mg/g AAE)	467 ± 13[a]	418 ± 53[a]	670 ± 14[b]	ND	ND	ND
(mg/g TE)	706 ± 30[b]	613 ± 44[a]	1,010 ± 52[c]	ND	ND	ND
IC_{50} of DPPH scavenging activity (µg/ml)	4.08	7.09	1.56	ND	1.95	1.12
α-amylase inhibition (%) at 35 µg/ml	17.6 ± 2.0[a]	53.0 ± 7.1[b]	63.8 ± 0.2[c]	49.0 ± 3.9[b]	ND	ND

Note: Data are presented as ± *SD* of triplicate. Different letters "a," "b," and "c" superscript means value of one treatment group is significantly ($p < .05$) different from the other for a given parameter or in vitro analysis.

Abbreviations: AAE, ascorbic acid equivalent; ACE, acetone extract; ETE, ethanol extract; GAE, gallic acid equivalent; IC_{50}, inhibition concentration (µg/ml) required to cause 50% inhibition; ND, not determined; TE, Trolox equivalent; WTE, water extract.

HPLC analysis on ACE showed the presence of ferulic acid, gallic acid, caffeic acid, vanillic acid, epicatechin, vanillin, and quercetin (Figure 1 and Table 2). Based on the percentage peak areas of the HPLC chromatogram (Figure 1 and Table 2), it appears that ferulic acid (29.3%), gallic acid (5.8%), and caffeic acid (4.3%) were the most abundant compounds identified, which may influence the polyphenol content and activity of ACE. Additionally, phenolic acids (ferulic acid, gallic acid, and caffeic acid) identified were more and had higher percentage peak areas compared to quercetin (1.8%), an identified flavonoid (Table 2), which correlates with the higher polyphenol than flavonoid contents in the extract (Table 1). However, in Tunisian grown pomegranate, ellagic acid, gallagic acid, punicalagin and derivatives, among others were reported in the acetone extract of the fruit peel (Abid et al., 2017), which may also influence antioxidant and antidiabetic properties.

3.2 | In vitro and cellular antioxidant activity of extracts

Oxidative stress is pivotal in the progression and complication of several diseases, including diabetes (Chukwuma & Islam, 2017). ROS and free radicals are highly reactive and molecules that oxidatively react and damage biological molecules, thus, leading some complications associated with several diseases (Pourghassem-Gargari et al., 2011). Physiological antioxidants, including vitamin E, vitamin C and reduced glutathione are known to suppress this process, by quenching the action of prooxidants and scavenging free radical. On the contrary, plants are endowed with polyphenols, which are good antioxidant due to their electron-donating ability (Chukwuma et al., 2019).

In this study, a dose dependent DPPH scavenging activity (IC_{50} = 1.56 μg/ml) was shown by the ACE, which was comparable to ascorbic acid (IC_{50} = 1.95 μg/ml) and Trolox (IC_{50} = 1.12 μg/ml) and markedly more potent than the WTE (IC_{50} = 4.08 μg/ml) and ETE (IC_{50} = 7.09 μg/ml) (Table 2). Furthermore, regardless of the standard equivalent (gallic acid or ascorbic acid or Trolox) used, the FRAP of the ACE was significantly ($p < .05$) stronger than that of WTE and ETE (Table 1). Interestingly, when investigated for its nitric oxide radical-scavenging activity, the ACE (IC_{50} = 42.1 ± 9.1 μg/ml) significantly ($p < .05$) outperformed ascorbic acid (IC_{50} = 216 ± 16 μg/ml) and was comparable to Trolox (IC_{50} = 48.2 ± 17.3 μg/ml) (Figure 2 and Table 3), which suggests a remarkable antioxidant potential of *P. granatum* fruit peel ACE.

The potent antioxidant property of *P. granatum* fruit peel acetone extract relative to other organic and water extracts have been documented. Thitipramote et al. (2019) reported a significantly ($p < .05$) higher DPPH scavenging and ferric reducing activities of *P. granatum* fruit peel acetone extract compared to the water and ethanol extracts. Karthikeyan and Vidya (2019) reported that *P. granatum* fruit peel acetone extract showed DPPH scavenging activity that was 2.7

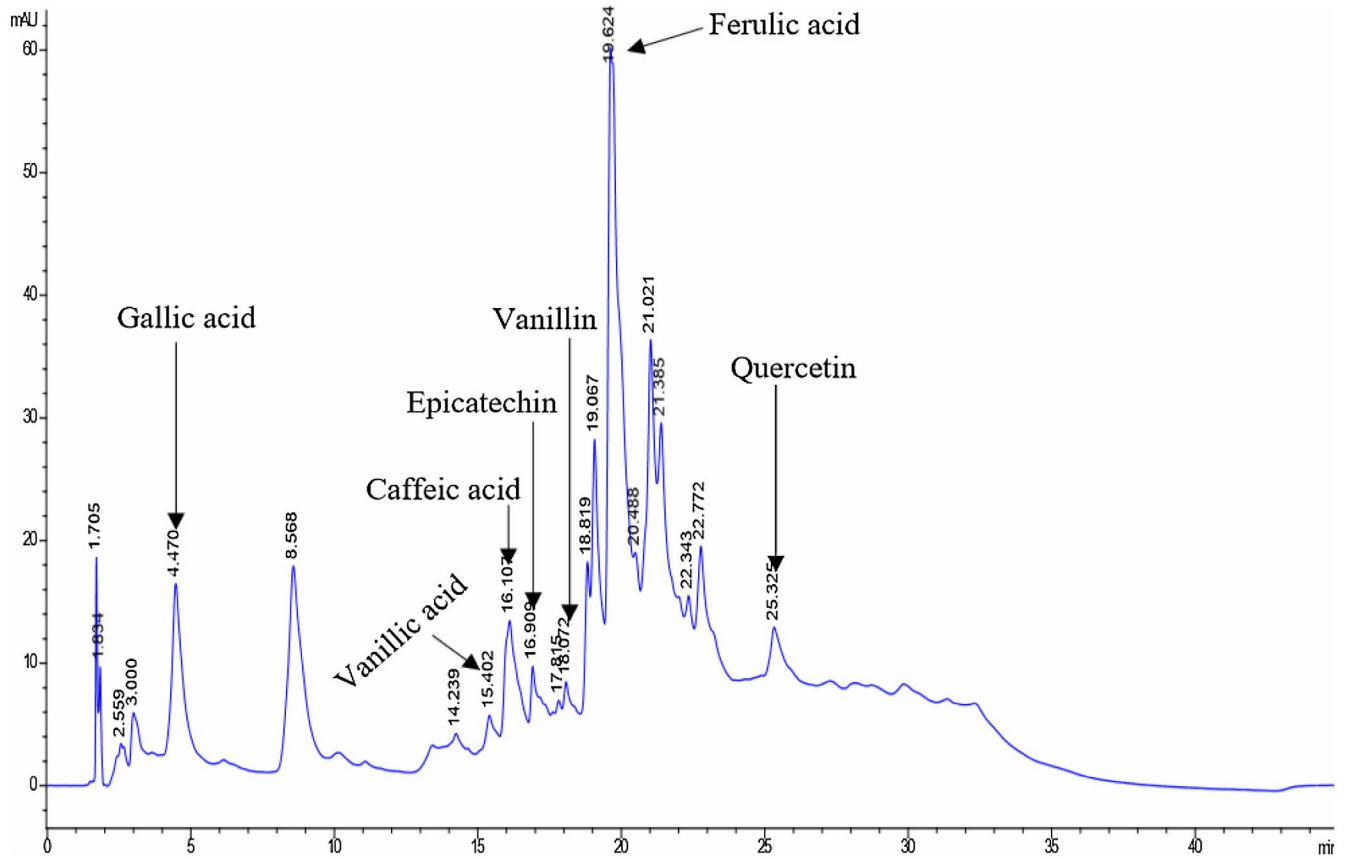

FIGURE 1 HPLC fingerprint of ACE showing the presence of gallic acid, vanillic acid, caffeic acid, epicatechin, vanillin, ferulic acid, and quercetin

TABLE 2 Retention times and percentage peak areas of HPLC-Identified polyphenols in the ACE of *P. granatum* fruit peel

Polyphenols	Parameters	ACE
Quercetin (RT: 25.21 min)	RT (min)	25.325
	DFS (min)	+0.115
	Peak area (%)	1.8115%
Ferulic acid (RT: 19.637 min)	RT (min)	19.624
	DFS (min)	−0.013
	Peak area (%)	29.3050%
Gallic acid (RT: 4.458 min)	RT (min)	4.470
	DFS (min)	+0.012
	Peak area (%)	5.8442%
Caffeic acid (RT: 15.997 min)	RT (min)	16.107
	DFS (min)	+0.11
	Peak area (%)	4.2589%
Vanillic acid (RT: 15.517 min)	RT (min)	15.402
	DFS (min)	−0.115
	Peak area (%)	0.7069%
Vanillin (RT: 18.134 min)	RT (min)	18.072
	DFS (min)	−0.062
	Peak area (%)	0.5874%
Epicatechin (RT: 16.83 min)	RT (min)	16.909
	DFS (min)	+0.079
	Peak area (%)	0.7334%

Abbreviations: DFS, the difference between the peak retention time of the extract and standard; RT, peak retention time.

and 1.6 times and ferric reducing activity that was 1.3 and 1.9 times stronger that the water and hexane extracts. The potent antioxidant property of the ACE may be attributed to the abundant phenolic acids (ferulic acid, gallic acid, and caffeic acid) and the presence of quercetin (Figure 1 and Table 2), which are known phytopolyphenols with impressive antioxidant properties (Piazzon et al., 2012; Rice-Evans, Miller, & Paganga, 1997).

In vitro studies on lard (pig fat) have shown that *P. granatum* fruit peel acetone extract inhibited lipid peroxidation and outperformed the water, methanol, and ethyl acetate extract as well as Butylated hydroxytoluene, a known antioxidant (Zhang, Jia, & Yao, 2007). Consistent data in the present showed that in Chang liver cells, the ACE inhibited oxidative stress-induced lipid peroxidation elevation (IC_{50} = 62.2 ± 16.1 μg/ml) and reduced glutathione reduction (IC_{50} = 156 ± 54 μg/ml) (Figure 2b,c and Table 3). The inhibitory effect of ACE on both lipid peroxidation elevation and reduced glutathione reduction was statistically comparable to that of ascorbic acid (IC_{50} = 30.2 ± 8.8 and 65.4 ± 3.8 μg/ml, respectively) and gallic acid (IC_{50} = 46.2 ± 20.9 and 77.5 ± 29.6 μg/ml, respectively) (Figure 2B,C and Table 3).

Elevated lipid peroxidation and depleted GSH level are classical conditions depicting oxidative stress. These conditions result in high physiological levels of ROS and free radicals, which cause oxidative damage to tissues (Chukwuma et al., 2017). Thus, the ability of the ACE to suppress lipid peroxidation elevation and GSH depletion in hepatocytes suggests potential protective effect against oxidative damage in organs and associated complications. This effect may be influenced by the observed notable presence of ferulic acid, caffeic acid, and gallic acid in the ACE (Figure 1 and Table 2), as well as punicalagin and derivatives, which have been reported as a major constituent of *P. granatum* fruit peel acetone extract (Abid et al., 2017). While these phenolic have been reported to suppress lipid peroxidation and free radical production on iron- and diabetes-induced oxidative stress (Balasubashini, Rukkumani, Viswanathan, & Menon, 2004; Kristinová, Mozuraityte, Storrø, & Rustad, 2009; Punithavathi, Stanely Mainzen Prince, Kumar, & Selvakumari, 2011), a recent study has shown that punicalagin reduces hepatic lipid peroxidation in type 2 diabetic rats (El-Beih, Ramadan, El-Husseiny, & Hussein, 2019).

3.3 | In vitro inhibition of glycation process and carbohydrate and lipid digestive enzymes

The rate of carbohydrate and lipid digestion can influence or adversely affect postprandial glycaemia, weight gain, and obesity, particularly in people at risk of T2D. Elevated release of non-saturated fatty acids is often observed in type 2 diabetes and in obesity, which has been linked to insulin resistance in both conditions (Al-Goblan, Al-Alfi, & Khan, 2014). Hence, limiting carbohydrate and lipid digestion is considered a therapeutic approach to control hyperglycemia, weight gain, and obesity (Birari & Bhutani, 2007; Oyebode et al., 2018). Antidiabetic drugs like the α-glucosidase inhibitors and antiobesogenic drugs like the pancreatic lipase inhibitors exert their therapeutic effects using this mode of action.

Previous studies have reported the inhibitory effects of the hydroalcoholic extracts of *P. granatum* fruit peel and leaves on α-amylase activity, lipase activity, and lipid digestion (Adnyana et al., 2014; Hasan et al., 2018; Jain et al., 2012; Yu et al., 2017), with scarce information on the acetone extract. In the present study, preliminary data showed that the ACE of *P. granatum* fruit peel (64%) showed a significantly ($p < .05$) higher α-amylase inhibition than the WTE (18%) and ETE (53%) (Table 1). Further studies on the α-amylase inhibitory potentials of the ACE showed it significantly ($p < .05$) outperformed acarbose (IC_{50} = 10.6 ± 2.8 vs. 19.2 ± 5.8 μg/ml, respectively) (Figure 3A and Table 3) in a dose dependent manner, which suggests that the ACE of *P. granatum* fruit peel may be an understudied extract containing potent α-amylase inhibitory principles that may afford glycemic control. The potent inhibitory effect of the ACE may be influenced by the abundance of ferulic acid (Figure 1 and Table 2), a phenolic acid with α-amylase inhibitory pharmacological credence (Jeong, Cho, & Lee, 2012). On the contrary, the ACE did not show promising inhibition of pancreatic lipase activity, relative to orlistat (Figure 3B and Table 3), suggesting it may not be a preferred source of anti-lipase principle.

Glycemic control in diabetic can help to suppress hyperglycemia-induced protein and lipid glycation, a process that results in the formation of advance glycated end products (AGEs) formation,

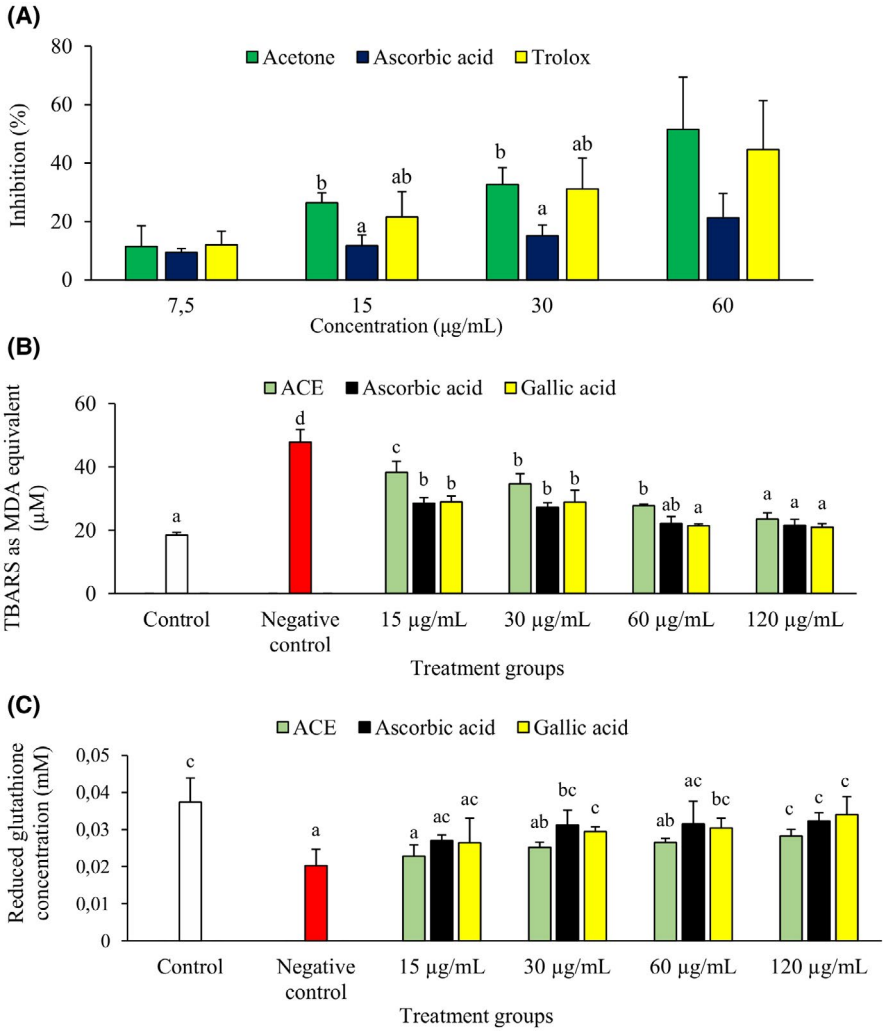

FIGURE 2 The effect of the ACE of *P. granatum* fruit peel on (A) in vitro NO radical-scavenging, as well as (B) lipid peroxidation, and (C) reduced glutathione (GSH) in Chang liver cell induced with oxidative stress. Data are presented as mean ± *SD* of triplicate (for in vitro assays) or technical replicates to 2 biological repeats (for cellular studies). Different letters "a," "b," and "c" mean value of one treatment group is significantly ($p < .05$) different from the other for a given parameter or in vitro analysis

which worsens diabetes complications due to oxidative damage in organs and tissues (Cooper, 2004). Interestingly, in addition to the excellent α-amylase inhibitory glycemic control potential of ACE, it showed a dose dependent but moderate antiglycation activity (IC_{50} = 16.2 ± 5.6 µg/ml), with 61% inhibition at 80 µg/ml (Figure 3C and Table 3). These data suggest that *P. granatum* fruit peel may contain bioactive principle that may be useful in the management or prevention of diabetic complications.

3.4 | Effect the ACE on Chang liver cell glucose uptake/utilization

Following the impressive in vitro antidiabetic and antioxidative activity of the ACE, it was further subjected to glucose uptake study using Chang liver cell. Glucose uptake in the liver is one of the body's glycemic control mechanisms, in which circulating glucose is taken up and utilized or stored as glycogen in the liver (Aronoff, Berkowitz, Shreiner, & Want, 2004). Biguanides (e.g., metformin), a class of antidiabetic are antagonistic toward hepatic glucose release, thus, preserving hepatic glucose uptake and storage (Bailey, 1992). Although not as potent as metformin, the ACE showed a dose dependent increase in hepatocyte glucose uptake (EC_{50} = 60.3 ± 25.0 µg/ml) relative to the control (without ACE treatment) (Figure 4 and Table 3), which suggests that the bioactive principle of this extract may potentiate glycemic control via hepatic glucose utilization and/or storage.

3.5 | Cell growth inhibition of the ACE of *P. granatum* fruit peel

For the purpose of safety, ascertaining the toxicity profile of therapeutic agent is an important step in drug discovery. In the present study, the effect of the ACE of *P. granatum* fruit peel on the growth or viability of liver (Chang liver cell line) and kidney (Vero cell line) cells was used as an in vitro model to investigate the toxicity profile

of this extract. These cell lines were selected for this study because they are building unit of two key metabolic organs (liver and kidney) in the body. While the ACE showed proliferative tendencies on the kidney cells, it did not detrimentally affect the viability or growth of the liver cells, with IC_{50} (> 10,000 μg/ml) far higher than the bioactive doses of the extract (Figure 5 and Table 3). These results suggest

TABLE 3 IC_{50} or EC_{50} values (μg/ml) for the activity of the ACE of *P. granatum* fruit peel in different bioassays

Parameters or activity	ACE	Ascorbic acid	Trolox	Gallic acid	Aminoguanidine	Acarbose	Orlistat
	IC_{50} or EC_{50} values (μg/ml)						
NO scavenging activity (IC_{50})	42.1 ± 9.1[a]	216 ± 16[b]	48.2 ± 17.3[c]	ND	ND	ND	ND
Antiglycation activity (IC_{50})	16.2 ± 5.6[a]	ND	ND	ND	2.31 ± 0.85[b]	ND	ND
α-amylase inhibition (IC_{50})	10.6 ± 2.8[a]	ND	ND	ND	ND	19.2 ± 5.8[b]	ND
Pancreatic lipase inhibition (IC_{50})	53.3 ± 6.3[a]	ND	ND	ND	ND	ND	2.49 ± 0.99[b]
Chang liver cell lipid peroxidation inhibition (IC_{50})	62.2 ± 16.1	30.2 ± 8.8	ND	46.2 ± 20.9	ND	ND	ND
Inhibition of Chang liver cell GSH level reduction (IC_{50})	156 ± 54	65.4 ± 3.8	ND	77.5 ± 29.6	ND	ND	ND
Chang liver cell glucose uptake (EC_{50})	60.3 ± 25.0	ND	ND	ND	ND	ND	ND
Cell growth inhibition (IC_{50})							
Kidney cell	ND	ND	ND	ND	ND	ND	ND
Liver cell	>10,000	ND	ND	ND	ND	ND	ND

Note: Data are presented as ± *SD* of triplicate (for in vitro assays) or technical replicates to 2 biological repeats (for cellular studies). Different letters "a," "b," and "c" superscript mean value of one treatment group is significantly ($p < .05$) different from the other for a given parameter or in vitro analysis.

Abbreviations: ACE, acetone extract of *P. granatum* fruit peel; EC_{50}, effective concentration (μg/ml) required to cause 50% of effect; GSH, reduced glutathione; IC_{50}, inhibition concentration (μg/ml) required to cause 50% inhibition; ND, not determined.

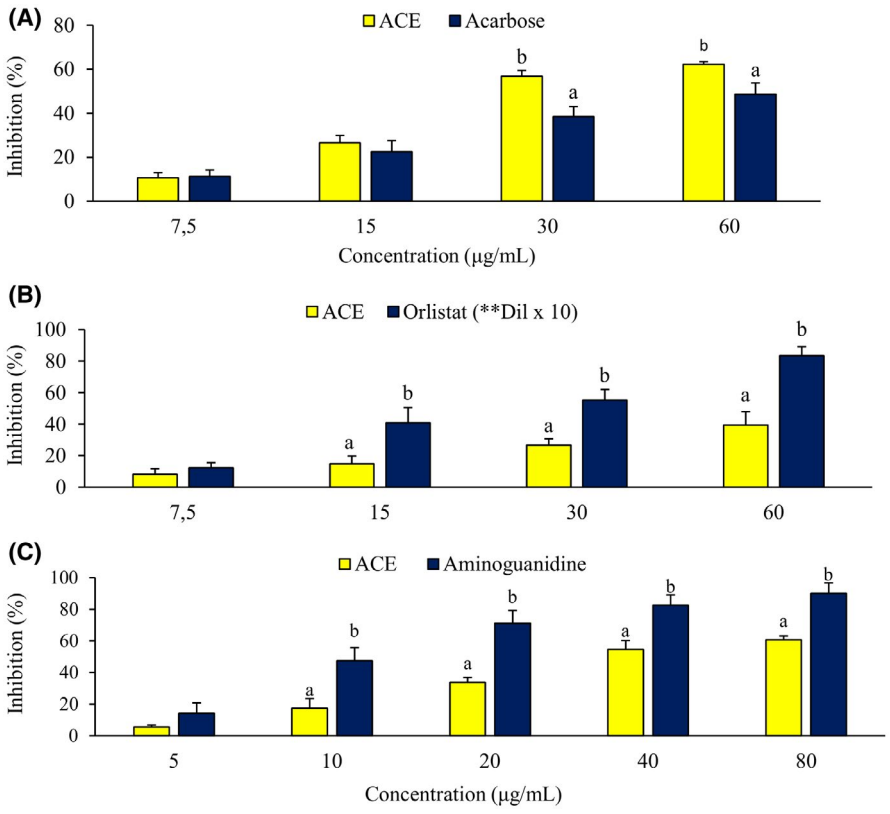

FIGURE 3 The in vitro inhibitory effect of the ACE of *P. granatum* fruit peel on (A) α-amylase activity (B) pancreatic lipase activity (**Dil × 10 means each concentration of Orlistat is 10 × diluted; 0.75–6 μg/ml), and (C) BSA glycation. Data are presented as ± *SD* of triplicates. Different letters "a" and "b" mean value of one treatment group is significantly ($p < .05$) different from the other for each concentration

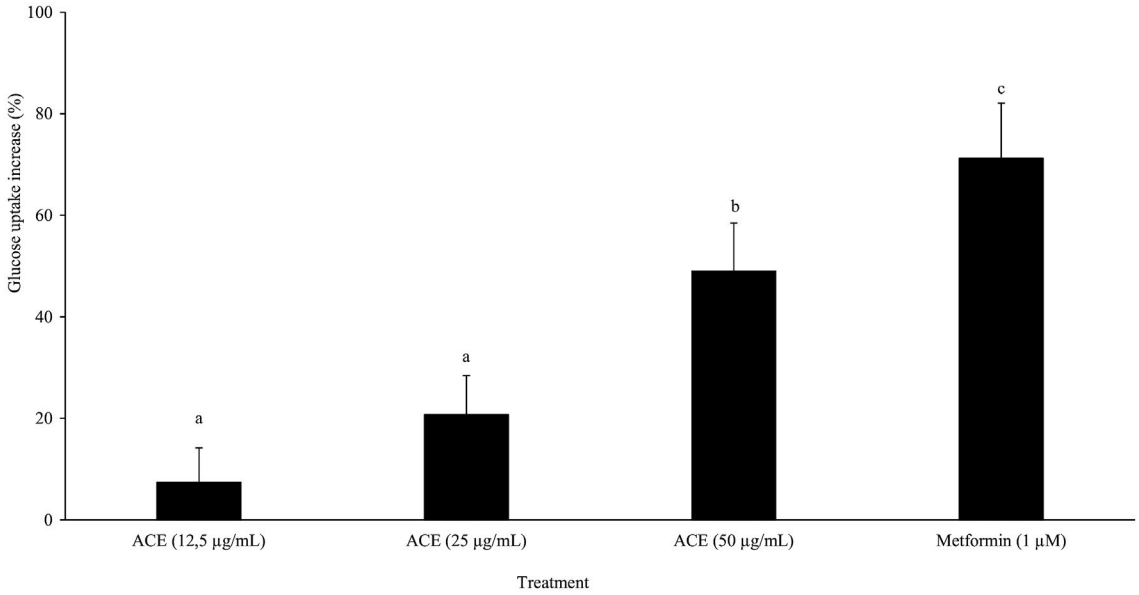

FIGURE 4 Glucose uptake increase in Chang liver cells following treatment with the ACE of *P. granatum* fruit peel. Data are presented as ± *SD* of technical replicates to 2 biological repeats. Different letters "a," "b," and "c" mean value of one treatment group is significantly ($p < .05$) different from the other for a given in vitro analysis

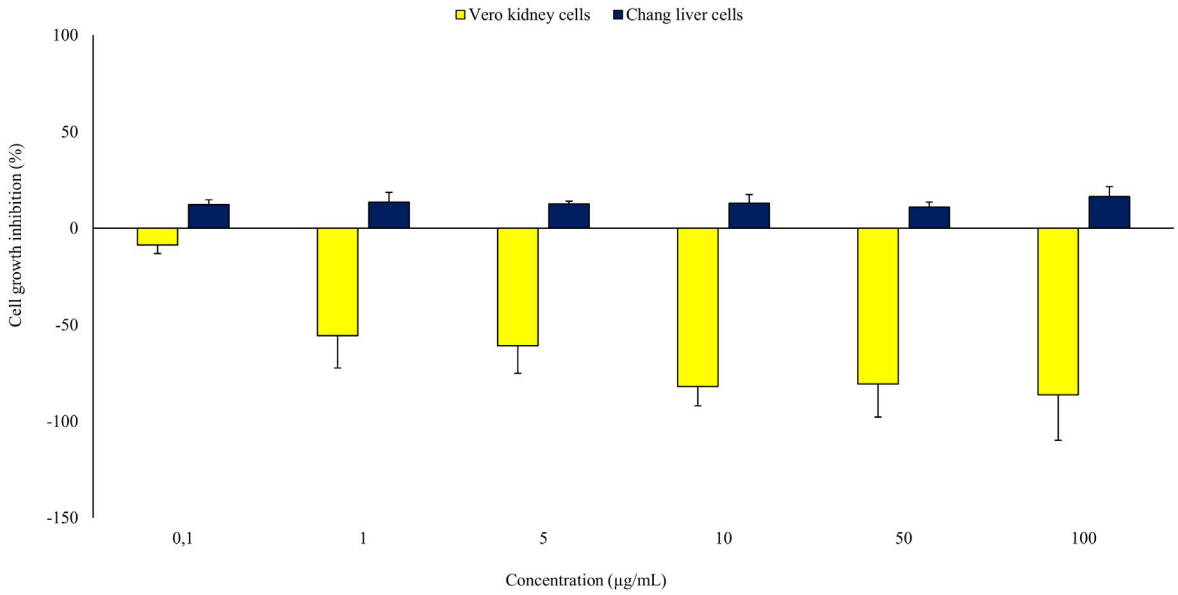

FIGURE 5 Cell growth inhibition of the ACE of *P. granatum* fruit peel. Data are presented as ± *SD* of technical replicates to 2 biological repeats

that the ACE of *P. granatum* fruit peel may not contain toxic bioactive principles, and thus, may be a safe extract for further in vivo studies.

4 | CONCLUSION

Over the years, pharmacological studies on the different parts of *P. granatum*, particularly the fruits and leaves have mostly explored the hydroalcoholic extracts of this plant. Data of the present study showed that the acetone extract of the fruit peel has more phenolic and flavonoid content and possess stronger antioxidant and α-amylase inhibitory activities compared to the hydroalcoholic extracts. Further studies showed that the acetone extract increased glucose uptake and improved oxidative stress in hepatocytes, which may be influenced by its richness in some bioactive phenolic acid. Oxidative stress remains pivotal in the progression and complication of diabetes; thus, the dual acting antidiabetic and antioxidative potentials of the acetone extract suggest that it may afford glycemic control by suppressing carbohydrate digestion and increasing

hepatic glucose utilization, as well as concomitantly suppressing glycation and other prooxidative processes that may cause damage to tissues, such as the liver in diabetic condition. This study suggests that the acetone extract of *P. granatum* fruit peel may be an understudied extract that contains potent antidiabetic and antioxidative bioactive principles with minimal toxicity, and thus, may be a promising extract for diabetes and related oxidative adversities, following appropriate further studies.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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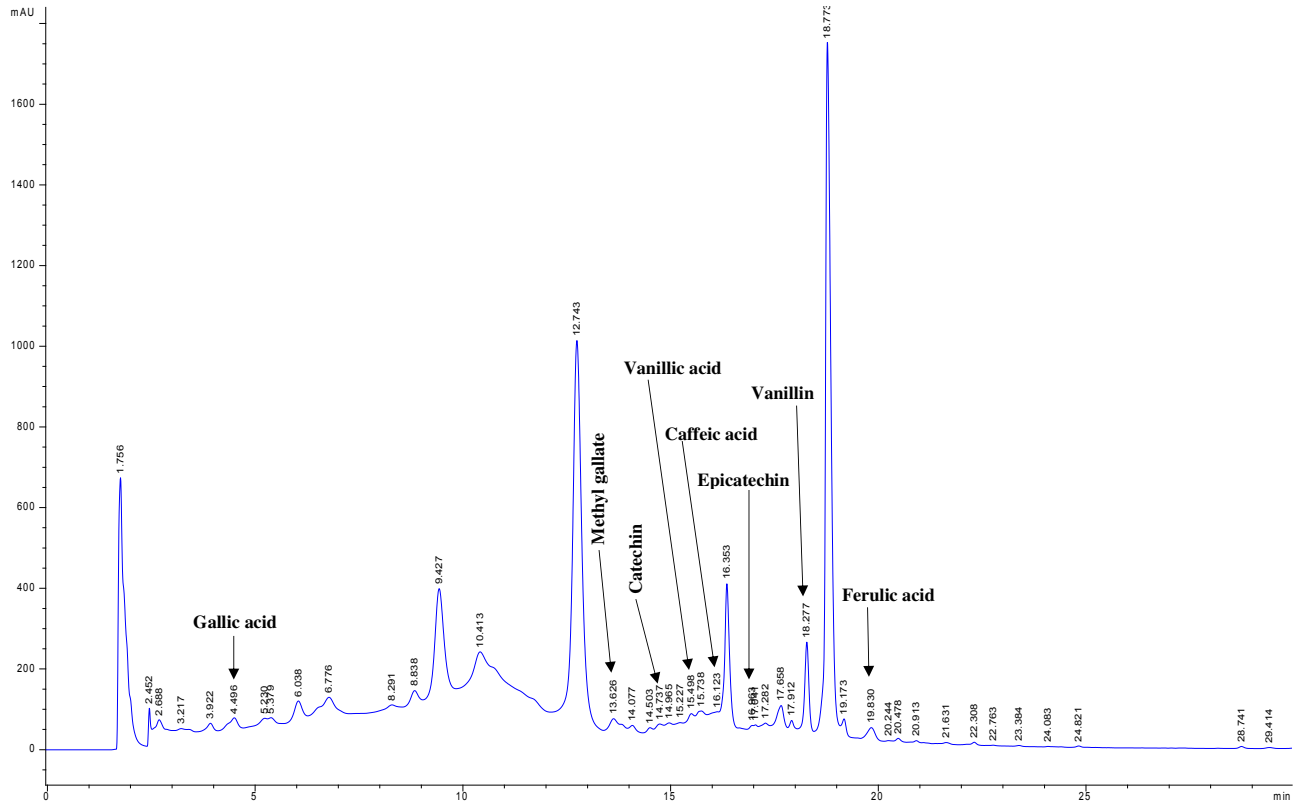
Corrigendum

Corrigendum to published article:

Chukwuma, CI, Mashele, SS, Akuru, EA. Evaluation of the in vitro α-amylase inhibitory, antiglycation, and antioxidant properties of *Punica granatum* L. (pomegranate) fruit peel acetone extract and its effect on glucose uptake and oxidative stress in hepatocytes. *J Food Biochem.* 2020; 44:e13175. https://doi.org/10.1111/jfbc.13175

This serves as a notification for the corrections being made in the above-mentioned article published in the Journal of food Biochemistry. After a careful re-review and re-proofreading of the referred published article, we noticed that we mistakenly and unintentionally analyzed and reported the wrong HPLC chromatogram (Figure 1) in the published version of the article. Since this error could affect the interpretation of data or reliability of information presented, we the authors are correcting this mistake and presenting the correct HPLC chromatogram, as well as the correct interpretation. The following corrections below are now being made in the corrected version of article:

1. Figure 1 is now replaced with the following figure or image below, which is the correct HPLC chromatogram:



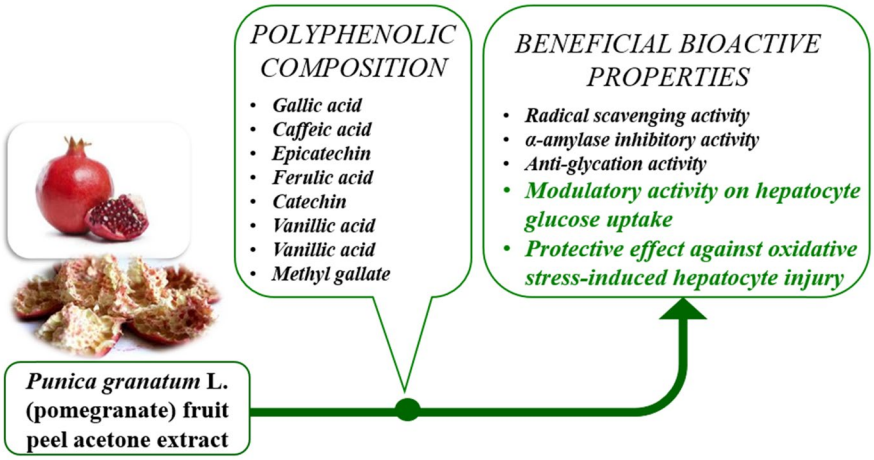
2. The caption of Figure 1 is now written as follows to correspond with the correct HPLC chromatogram in Figure 1: "*HPLC fingerprint of ACE showing the presence of gallic acid, vanillic acid, caffeic acid, epicatech, vanillin, ferulic acid, catechin and methyl gallate.*"

3. The table in "Table 2: Retention times and percentage peak areas of HPLC-Identified polyphenols in the ACE of *P. granatum* fruit peel" is now replaced with the following table below, which corresponds to the retention times, % peak areas and identified polyphenols of the correct HPLC chromatogram in Figure 1:

Polyphenols	Parameters	Ace
Catechin (RT: 14.73 min)	RT (min)	14.737
	DFS (min)	+0.0.007
	Peak area (%)	0.6333%
Ferulic acid (RT: 19.637 min)	RT (min)	19.830
	DFS (min)	+0.193
	Peak area (%)	0.8044%
Gallic acid (RT: 4.458 min)	RT (min)	4.496
	DFS (min)	+0.038
	Peak area (%)	1.6531%
Caffeic acid (RT: 15.997 min)	RT (min)	16.123
	DFS (min)	+0.126
	Peak area (%)	1.0890%
Vanillic acid (RT: 15.517 min)	RT (min)	15.498
	DFS (min)	−0.019
	Peak area (%)	0.9608%
Vanillin (RT: 18.134 min)	RT (min)	18.277
	DFS (min)	+0.143
	Peak area (%)	1.8811%
Epicatechin (RT: 16.83 min)	RT (min)	16.963
	DFS (min)	+0.133
	Peak area (%)	0.3720%
Methyl gallate (RT: 13.81 min)	RT (min)	13.626
	DFS (min)	−0.184
	Peak area (%)	1.5181%

RT: Peak Retention time; DFS: The difference between the peak retention time of the extract and standard.

4. Abstract section: The sentence preceding the last sentence is now written as follows to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2: *"HPLC analysis showed the presence of bioactive phenolics (ferulic, caffeic, vanillic and gallic acids, vanillin, methyl gallate, epicatechin and catechin) in this extract"*
5. Graphical abstract: The graphical abstract is now replaced with the revised version to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2.



6. Section 3 (Result and discussion section)

   a. Subsection 3.1 (Yield and phytochemical characterization of extracts): Paragraph 2 is now written as follows to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2: *"HPLC analysis on ACE showed the presence of ferulic acid, gallic acid, caffeic acid, vanillic acid, epicatechin, epicatechin, vanillin and methyl gallate (Figure 1 and Table 2). Although the percentage peak areas (0.34%–1.88%) of the identified compounds observed in the HPLC chromatogram (Figure 1 and Table 2) suggest that the identified compounds occurred in minute amounts, they may influence the polyphenol content and activity of ACE. On the other hand, although not identified, the major peaks observed in the chromatogram may be ellagitannin components (punicalagin, ellagic acid, gallagic acid etc), which have been documented as part of the predominant bioactive polyphenol constituents of pomegranate fruit (Abid et al., 2017). Moreover, ellagitannin components including punicalagin, ellagic acid, gallagic acid and derivatives, among others were reported in the acetone extract of the fruit peel of Tunisian grown pomegranate (Abid et al., 2017), which may also influence antioxidant and antidiabetic properties."*
   b. Subsection 3.2 (*In vitro* and cellular antioxidant activity of extracts): The last sentence of paragraph 1 is now written as follows to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2: *"The potent antioxidant property of the ACE may be attributed to the identified phenolics (ferulic acid, gallic acid, caffeic acid, catechin, vanillic acid etc) (Figure 1 and Table 2) and perhaps, the unidentified ellagitannin components, which are known phytopolyphenols with impressive antioxidant properties (Rice-Evans, Miller, & Paganga, 1997; Piazzon et al., 2012; Middha et al., 2013; Abid et al., 2017; Khalil et al., 2017; 2018)."*
   c. Subsection 3.3 (*In vitro* inhibition of glycation process and carbohydrate and lipid digestive enzymes): The sentence preceding the last sentence of paragraph 2 is now written as follows with a new citation and/or reference (*Roškar, I., Štrukelj, B., & Lunder, M. (2016) Screening of Phenolic Compounds Reveals Inhibitory Activity of Nordihydroguaiaretic Acid Against Three Enzymes Involved in the Regulation of Blood Glucose Level. Plant Foods for Human Nutrition, 71(1), 88-89*) added to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2: *"The potent inhibitory effect of the ACE may be influenced by the identified phenolic compounds (Figure 1 and Table 2), which have been reported to have α-amylase inhibitory pharmacological credence (Jeong, Cho, & Lee, 2012; Roškar, Štrukelj , & Lunder, 2016)."*
7. Conclusion: The following phrase, "……..improved oxidative stress in hepatocytes, which may be influenced by its richness in some bioactive phenolic acid," is now written as follows to correspond with the correct interpretation of the correct HPLC data in Figure 1 and Table 2: *"…….improved oxidative stress in hepatocytes, which may be influenced by the presence some bioactive phenolic compounds."*
8. Supplementary data: The HPLC chromatogram of quercetin standard in Supplementary 6 is now replaced with the HPLC chromatogram of catechin standard, while the HPLC chromatogram of methyl gallate standard is now included as Supplementary 8.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.