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Original article

Profiling the phyto-constituents of *Punica granatum* fruits peel extract and accessing its *in-vitro* antioxidant, anti-diabetic, anti-obesity, and angiotensin-converting enzyme inhibitory properties

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ABSTRACT

This context was investigated to assess the *in vitro* antioxidant, anti-diabetic, anti-obesity, and angiotensin-converting enzyme (ACE) inhibition traits of *Punica granatum* fruits peel extract. Initially, among various extracts tested, aqueous and ethanolic peel extracts depicted the presence of diverse phytoconstituents. *In vitro* antioxidative properties of peel extracts were determined using standard methodologies. Results showed that aqueous and ethanolic extracts had IC_{50} values of 471.7 and 509.16 μg/mL, respectively in terms of 1,1,diphenyl 2,2,picrylhydrazyl scavenging. Likewise, IC_{50} values of aqueous and ethanol extract were obtained as 488.76 and 478.47 μg/mL towards the degradation of hydrogen peroxide. The ethanolic extract exhibited the highest inhibition of α-glucosidase by showing activity of 53.34 ± 2.0 to 15.18 ± 1.4 U/L in a dose dependent manner (100–1000 μg/mL). Ethanolic extract was reported as the most active inhibitor of lipase with an IC_{50} value of 603.50 μg/mL. Ethanolic extract showed increased inhibition of ACE in a concentration dependent manner (100–1000 μg/mL) with IC_{50} value of 519.45 μg/mL. Fourier transform-infrared spectrum revealed the availability of various functional groups in the ethanolic extract of peel. Gas chromatography-mass spectrometry chromatogram of peel extract illustrated 23 diversified chemical constituents including 1,2,3,4-butanetetrol, Dimethyl sulfone, 9-octadecenamide, and Pentadecanoic acid as predominant compounds. In summary, *P. granatum* fruits peel extract revealed promising antioxidant, anti-diabetic, anti-obesity, and anti-hypertensive properties.

1. Introduction

Diabetes mellitus (DM) is a major chronic disease with persistent hyperglycaemia which is affecting global population at an alarming rate. In 2019, the global DM prevalence is estimated to be 9.3% (463 million people) and the total count is predicted to rise by 2045 (Saeedi et al., 2019). Diabetes mellitus can deteriorate various organ systems due to the metabolic aberrations and immune dysfunction which can lead to various complications such as retinopathy, nephropathy, neuropathy, and increased risk of cardiovascular diseases (Goyal and Jialal, 2020). The prevalence of co-morbidities like hypertension and obesity are seen commonly

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among people with DM (Chaudhary et al., 2019). In DM, immune dysfunction occurring in a hyperglycaemic environment leads to the greater frequency of bacterial infections associated with acute and emphysematous pyelonephritis, abscess, gangrene, foot infections, and external otitis as well as fungal infections associated with cystitis and candidiasis (Casqueiro et al., 2012). Without integrated pharmaceutical and medical nutrition therapy, DM could be disabling, life-threatening, and expensive.

Hypertension is another common progressive disorder worldwide which leads to varied chronic diseases. Angiotensin converting enzyme (ACE) produced from the lungs converts angiotensin I into angiotensin II which causes vasoconstriction followed by hypertension. At present, antioxidants are widely used for their preventive role against cardiovascular diseases as well as potentiality for scavenging free radicals (Nwaji et al., 2016; Jäkälä and Vapaatalo, 2010). A huge population of the world is affected by hypertension, and this metabolic disorder is expected to increase globally in future (Mittal and Singh, 2010). In spite of adopting life style changes (exercise and healthy diets) as common preventive approaches, the administration of drugs is imperative at critical stages. Hence, there is urgency to prevent and cure hypertension associated disorders by identifying natural therapeutics.

Phytochemicals offer wide varieties of therapeutically active compounds, which are considered less toxic, safer, and cheaper with better drug delivery and therapeutic properties compared to synthetic compounds (Khusro et al., 2013; Al-Dhabi et al., 2015; Al-Dhabi and Arasu, 2016; Barathikannan et al., 2016; Park et al., 2016a; Park et al., 2016b; Cuong et al., 2017; Ilavenil et al., 2017; Parveen et al., 2018; Esther Lydia et al., 2019). *Punica granatum* or pomegranate belongs to Lythraceae family. Constituents of *P. granatum* fruits and its peel are known to depict varied biological properties (Barbosa-Filho et al., 2006; Janani and Esther Lydia, 2013; Barathikannan et al., 2016; Esther Lydia et al., 2020). *P. granatum* peel has several phytochemicals like polyphenolic compounds, phenolic acids, anthocyanins, and flavonoids as potent antioxidants (Middha et al., 2013; Madugula et al., 2017; Di Sotto et al., 2019).

Considering the pivotal therapeutic attributes of *P. granatum*, this investigation was aimed to demonstrate the *in vitro* antioxidant, anti-diabetic, anti-obesity, and ACE inhibitory traits of *P. granatum* fruits peel extract. Further, different types of bioactive components in *P. granatum* extract were identified using analytical assays.

2. Materials and methods

2.1. Collection of fruits

P. granatum fruits were procured from Pazhamudhir Nilayam, Chennai and authenticated. Peels of the fruits were manually removed and was spread on a muslin cloth for 5–6 days for drying. Fruits peels were then subjected to grinding for further processing.

2.2. Sequential extraction

Sequential extraction of fruits peel was performed as per the method of Esther Lydia et al. (2019) using acetone, ethanol, distilled water, petroleum ether, and chloroform as solvents. Solvents extracts were used for further experimental analyses.

2.3. Phytochemical analysis

The presence of varied phytocomponents (carbohydrate, flavonoid, tannins, saponins, alkaloids, quinines, glycosides, cardio glycoside, terpenoids, phenols, coumarins, steroids, phytosteroids, and anthraquinone) in various solvent extracts of peel was determined as per the method of Harborne (1993).

2.4. In vitro antioxidative activities-

2.4.1. 1, 1, diphenyl 2, 2, picrylhydrazyl (DPPH) scavenging assay

Among various extracts tested, the aqueous and ethanolic extracts were reported to be promising in terms of the presence of essential phytochemicals, and thus, only these two extracts were used for *in vitro* antioxidant activities and other analyses. The DPPH degrading properties of selected extracts of *P. granatum* peel at varied concentrations (100–1000 μg/ml) were determined as per the method of Shimada et al. (1992). Percentage (%) DPPH scavenging traits of peel extracts were calculated according to the equations as given below:

$$\text{DPPH scavenging } (\%) = [(A_{sample} - A_{blank})/ A_{control}] \times 100$$

2.4.2. Hydrogen peroxide (H_2O_2) degradation assay

Hydrogen peroxide scavenging traits of aqueous and ethanolic extracts of *P. granatum* peel at varied doses (100–1000 μg/mL) were estimated according to the methodology of Ruch et al. (1989) using the equation mentioned below:

$$\text{Hydrogen peroxide degradation } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.5. In vitro α-glucosidase activities

α-glucosidase activities of aqueous and ethanolic extracts were estimated as per the method of Esther Lydia et al. (2019).

2.6. In vitro anti-obesity test

Porcine pancreatic lipase (PPL type II) inhibitory properties of aqueous and ethanolic extracts of *P. granatum* peel were estimated following the methodology of Zheng et al. (2010). The inhibitory characteristic was estimated as per the equation mentioned below:

$$\text{Lipase inhibitory activity } (\%) = 1 - [(B - b)/(A - a) \times 100]$$

where, A - activity without the inhibitor, a - negative control without the inhibitor, B - activity with inhibitor, and b - negative control with inhibitor.

2.7. In vitro anti-hypertensive activities

Anti-hypertensive properties of extracts were determined in terms of ACE inhibition using UV–Vis spectrophotometer based on hippuric acid formation ability from hippuryl-L-histidyl-L-leusine (HHL) catalyzed by ACE (Chaudhary et al., 2014). Angiotensin converting enzyme (50 μL; 25 mU/mL) was mixed with 50 μL of the test solution at 37 °C for 10 min. On the other hand, substrate (150 μL; 8.3 mM of HHL in 50 mM sodium borate buffer constituting 0.5 M sodium chloride, pH 8.2) was mixed with solution and kept for 30 min at room temperature. Further, 250 μL of 1 M HCl was added into the mixture to stop the reaction, followed by the addition of 0.5 mL of ethyl acetate and centrifugation of mixture at 800 *g* for 10 min. Upper component (0.2 mL) was collected and evaporated under vacuum. Hippuric acid was mixed with distilled water and read spectrophotometrically at 228 nm. Ramipril (3.6 ng/mL) was used as standard and ACE inhibition was estimated according to the equation given below:

$$\text{ACE inhibition } (\%) = 1 - [(A - B)/C \times 100]$$

where, A is the optical density at 228 nm with ACE but without inhibitor, B is the optical density of both ACE and inhibitor, C is the optical density without ACE and inhibitor.

2.8. Analytical assays-

2.8.1. Fourier transform infra-red (FT-IR) spectroscopy

The FT-IR spectrum of ethanolic extract was carried out according to the methodology of Khusro et al. (2014).

2.8.2. Gas chromatography-mass spectrometry (GC-MS)

The GC-MS spectrum of ethanolic extract was performed as per the procedure of Esther Lydia et al. (2019) and relative peak areas were calculated.

2.9. Statistical analyses

Experiments were performed in triplicate and values were represented as mean ± standard deviation.

3. Results

3.1. Phytocomponent analysis

Table 1 illustrates the presence of fundamental preliminary phytochemical components in different solvent extracts of *P. granatum* peel. The investigation uncovered the availability of disparate phytochemicals in aqueous peel extract. Ethanolic extract was also reported rich in carbohydrates, tannins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, and steroids. In contrary, acetone extract demonstrated the availability of limited phytoconstituents while chloroform extract contained tannins, alkaloids, quinones, terpenoids, and steroids. Quinones and phenols were observed only phytochemicals present in petroleum ether extract.

3.2. Antioxidant activities

Fig. 1a shows the DPPH scavenging activities of peel extracts at different concentrations. At the highest concentration (1000 μg/mL), extracts showed DPPH radical degrading activities of 87.6 ± 1.8 and 78.32 ± 1.9%, respectively with respect to ascorbic acid (97.35 ± 1.6%). Result disclosed that aqueous extract, ethanolic extract, and ascorbic acid had an IC_{50} value of 471.70, 509.16, and 390.93 μg/mL, respectively.

The potential of *P. granatum* peel extracts to scavenge H_2O_2 is expressed in Fig. 1b. The extracts exhibited H_2O_2 degradation at distinct concentrations, however, the aqueous extract of fruit peel exhibited a high H_2O_2 restraint impact at various concentrations (100–400 μg/mL), while at 700 to 1000 μg/mL, ethanolic extract possessed a higher potential in degrading H_2O_2 with scavenging rate of 73.12 ± 1.7, 75.64 ± 1.6, 78.16 ± 1.7, and 80.68 ± 1.6%. IC_{50} values of aqueous and ethanolic extract, and standard were obtained as 488.76, 478.47, and 388.20 μg/mL, respectively.

3.3. α-glucosidase activities

The ethanolic extract depicted α-glucosidase inhibition by showing activity of 53.34 ± 2.0 to 15.18 ± 1.4 U/L in a concentration-dependent manner. In contrary, aqueous extract depicted activity of 65.48 ± 1.8 to 20.23 ± 1.3 U/L at varied concentrations tested (Fig. 2).

3.4. Anti-obesity properties

Fig. 3 illustrates lipase inhibitory potentials of aqueous and ethanolic extracts. With increase in concentrations, lipase inhibition also increased but the ethanolic extract was reported as the most active inhibitor of lipase with an IC_{50} value of 603.50 μg/mL.

3.5. Anti-hypertensive activities

The inhibition of ACE activity using different concentrations of the extracts is shown in Fig. 4. Ethanolic extract revealed increased inhibition of ACE at varied ranges (100–1000 μg/mL) with an IC_{50} value of 519.45 μg/mL with respect to the aqueous extract.

3.6. FT-IR spectrum

The FT-IR spectrum of ethanolic extract of *P. granatum* peel is shown in Fig. 5. The characteristic absorption band exhibited C—I stretching/halo compound, C—H bending/alkane, P—O—C stretching/aromatic phosphates, S=O stretching/sulfoxide/sulfone, C—O stretching/alkyl aryl ether, N—O stretching/Nitro compound, C=C stretching/ketone, C=O stretching/aldehyde, and N—H stretching/amine.

3.7. GC-MS chromatogram

GC-MS chromatogram of the peel extract is illustrated in Table 2, incorporating the retention time and area of bioactive components present. Among those bioactive components, 1,2,3, 4-butanetetrol (33.22%), Dimethyl sulfone (20.47%),

Table 1
Phytocomponents of *P. granatum* fruits peel extracts.

Phytochemicals	Aqueous extract	Ethanol extract	Acetone extract	Chloroform extract	Petroleum ether extract
Carbohydrates	+	+	+	–	–
Tannins	+	+	–	+	–
Saponins	+	+	–	–	–
Flavonoids	+	+	+	–	–
Alkaloids	+	+	+	+	–
Quinones	+	+	–	+	+
Glycosides	–	+	+	–	–
Cardiac glycosides	+	+	+	–	–
Terpenoids	+	+	–	+	–
Phenols	+	+	–	–	+
Coumarins	+	+	+	–	–
Steroids	+	+	–	+	–
Phytosteroids	–	–		–	–
Anthraquinone	–	–	–	–	–

'+': present; '–': absent.

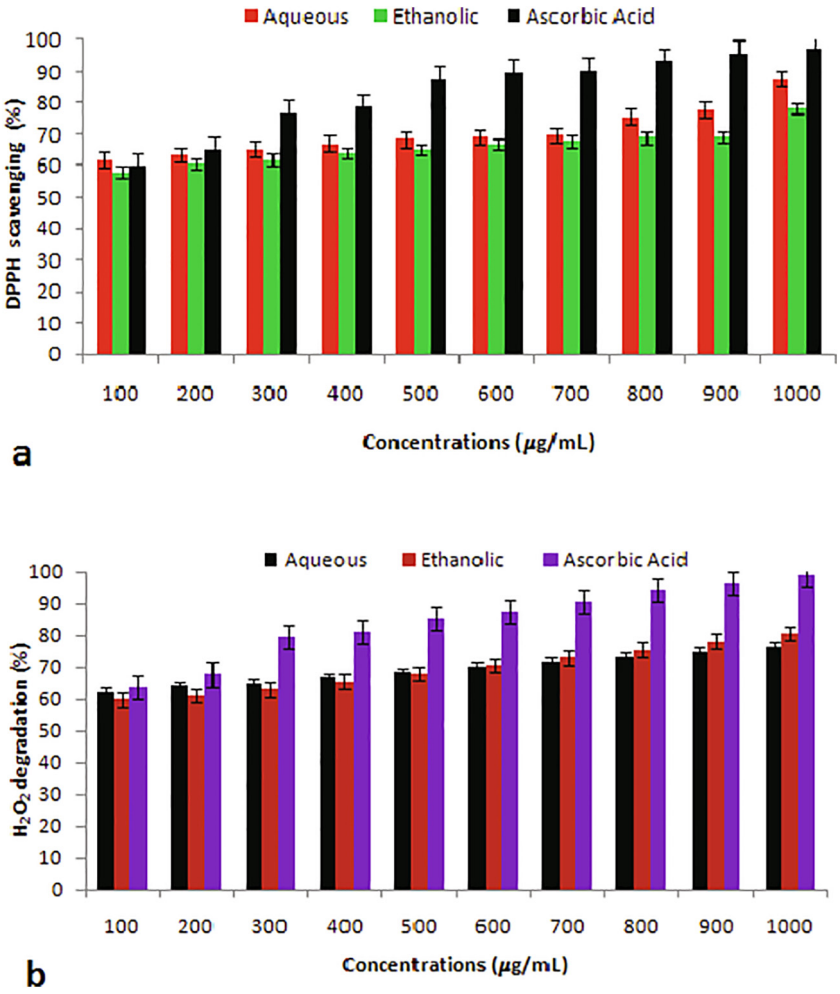

Fig. 1. (a) DPPH scavenging and (b) H_2O_2 degrading properties of peel extracts at various concentrations (100–1000 µg/mL).

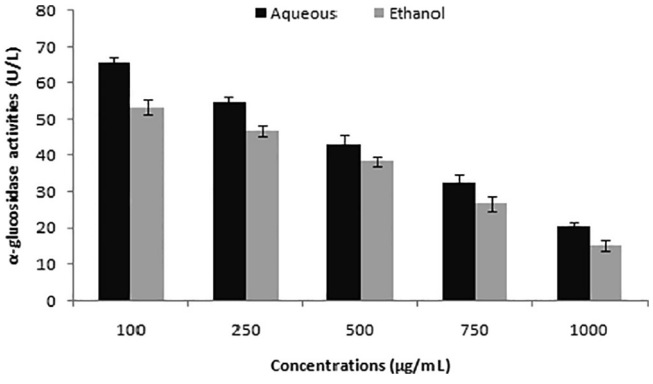

Fig. 2. α-glucosidase activities of peel extracts at various concentrations (100–1000 µg/mL).

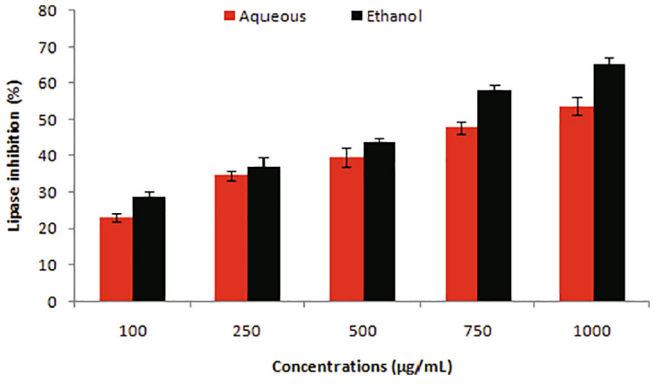

Fig. 3. Lipase inhibition traits of peel extracts at various concentrations (100–1000 µg/mL).

9-octadecenamide (13.14%), and Pentadecanoic acid (6.62%) were reported as the major compounds.

4. Discussion

In recent times, there is profound admiration in the estimation and utilization of medicinal plants as the well being of option/traditional medicine and some fundamental dietary supplements for maintaining several ailments relating to human health (Latha et al., 2019). The massive therapeutic significance of plants exclusively relies upon the bioactive composites, particularly phytochemicals that produce physiological effects on human health. In this regard, an alternative approach to diversiform chemical constituents of the medicinal plant becomes imperative to the scientific community to validate and document its medicinal applications just as precursors for organizing complex synthetic substances. Therefore, this investigation established a distinctive role of *P. granatum* fruits peel as adaptogenic and bio-therapeutics.

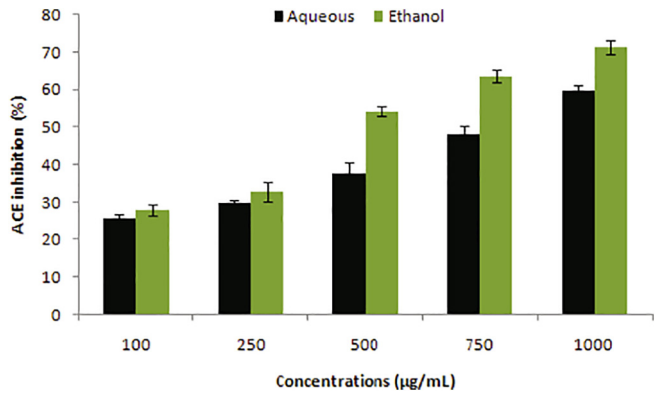

Fig. 4. ACE inhibition traits of peel extracts at various concentrations (100–1000 µg/mL).

The phytochemical analysis of the fruits peel extracts exhibited the availability of diversified components in different solvent extracts. Findings supported the reports of Bhandary et al. (2012) and Sangeetha (2015) who observed the availability of identical phytoconstituents in different extracts of *P. granatum* fruits peel. These phytoconstituents are known to offer imperative biological activities on physiological systems (Karthikeyan and Vidya, 2019).

At present, the emergence of synthetic oral anti-diabetic inhibitors has improved the performance in the management of DM, and its multifaceted complications as well as susceptibility to infections. But the regular use of these inhibitors comes with diluted effects. The present study revealed a protective efficacy of *P. granatum* fruits peel extracts against chronic hyperglycemia-induced oxidative stress linked to DM, thereby suggesting an approach to ameliorate its potential burden through the antioxidant application. In the antioxidant traits, aqueous and ethanolic extracts showed varied ranges of DPPH scavenging activities. The present findings agreed with the reports of Venkatadri et al. (2017) who illustrated pronounced degradation of DPPH at disparate concentrations of the extracts. However, this investigation uncovered that aqueous extract elicited the potent antioxidant activity, linking the fact that active phytochemicals of *P. granatum* fruits peel are readily dissolved in an aqueous medium. Plants rich in phytoconstituents, such as alkaloids, saponins, flavonoids, and phenols have been marked to protect the human body from free radicals, improves the normal metabolism of aerobic cells, and confer strong antioxidant defence mechanisms (Oboh et al., 2014). In this investigation, the DPPH degrading potential of aqueous extract suggests the availability of many hydroxyl groups in the structure of saponins as contained in the aqueous extract than ethanolic, acetone, chloroform, and petroleum ether extracts responsible for the enhancement of antioxidant activity in DM (Elekofehinti, 2015). Hydrogen peroxide deactivates enzymes by the oxidation of essential thiol (-SH) groups and reacts with Fe^{2+} similar to Cu^{2+} ions for forming hydroxyl radicals (Venkatadri et al., 2017). In this study, *P. granatum* peel extracts showed moderate scavenging of H_2O_2 which might be due to glycosides found in the extracts.

Diabetes mellitus is a multi-factorial disorder which causes several other complications such as diminished response of T-cells, neutrophil function, and humoral immunity disorders (Muller et al., 2005). Therefore, it demands multiple therapeutic approaches. α-glucosidase aids the digestion of dietary carbohydrates and starches to produce glucose for intestinal absorption, which thus prompts upsurge in blood glucose concentrations. In diabetics, this enzyme inhibition leads to delayed glucose absorption and lowering of postprandial hyperglycemia. In fact, Ngozi and Olatunbosun (2016) reported that α-glucosidase inhibition partially diminished the degree of HbA_{1c}. In a similar manner, α-glucosidase inhibitors suppressed postprandial hyperglycemia in DM (Lordan et al., 2013). In the present study, ethanolic extract was observed as the most active agent to inhibit α-glucosidase with respect to the aqueous extract. The high α-glucosidase inhibitory trait might be because of saponins present in the extract. Saponins have been found to play a significant impact in the cause of diabetic complications, and its hypoglycemic effect is mediated by distinct mechanisms, particularly the restoration of insulin response, α-glucosidase activity inhibition, and gluconeogenesis inhibition (Elekofehinti, 2015). Furthermore, the prominent α-glucosidase inhibition characteristic of extracts might also be because of phenols and flavonoids. Wang et al. (2004) established

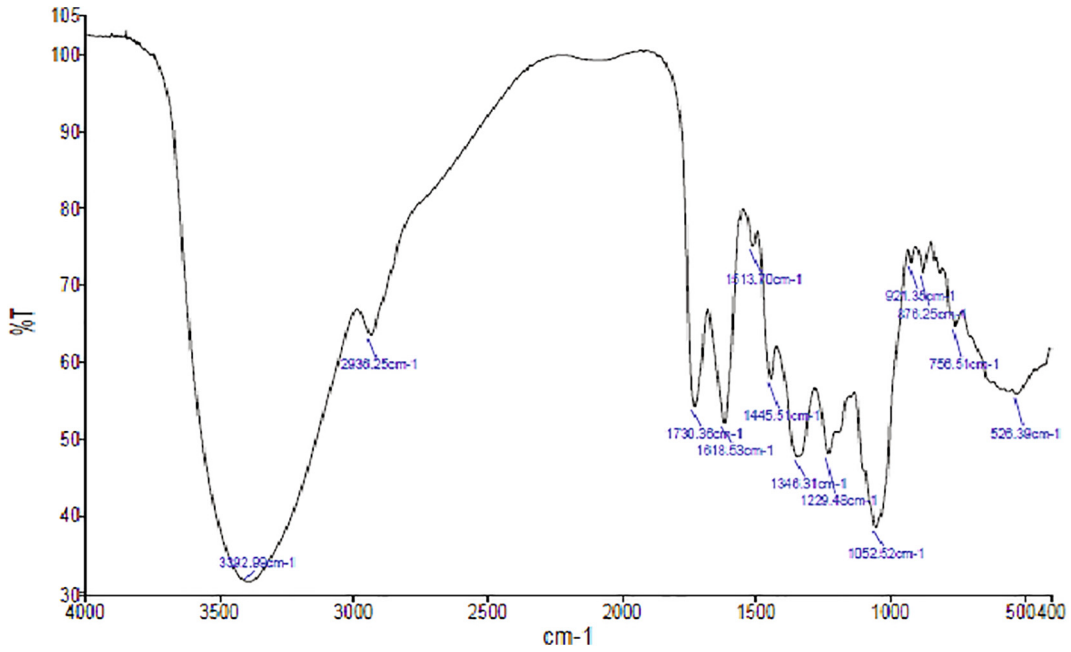

Fig. 5. FT-IR spectrum of ethanolic extract of *P. granatum* fruits peel.

Table 2
List of various compounds present in ethanolic extract of *P. granatum* fruits peel.

Peak	Retention time	Area (%)	Compound name
1	7.341	20.47	Dimethyl sulfone
2	7.452	33.22	1,2,3,4-butanetetrol, Erythritol
4	7.645	1.53	Nickel (II) bis(N,N-dioctyldithiocarbamate)
5	7.727	2.26	S-methyl methanethiosulphonate, Methyl 2-hydroxyethyl sulfoxide
6	8.091	1.46	Ethanol, 2,2′-[1,2-phenylenebis[(2-chloro-2,1-ethadediyl)oxy-2,1-ethanediyloxy]] bis-Thiohypophosphoric acid
7	8.239	1.86	Methane, (Methylsulfinyl) (Methylthio)-3,5-dithiahexanol 5,5-dioxide p-dioxane-2,3-diol
8	8.388	1.56	Dimethyl sulfoxide, 5,6-dihydro-4H-1-benzazonine-2,7-dione
9	8.470	1.49	2,2′-sulfinyldiethanol
10	8.507	0.81	2,2′-sulfinyldiethanol propanamide, 2-hydroxy-1-ethanol
11	8.603	1.63	2-chloroethyl thiocyanate butane, 2,3-dichloro-dimethyl sulfoxide
12	8.700	2.25	3,7-octadiene-1,1,8-tricarboxylic acid, 3,7-dimethyl, trimethyl ester, 1,4-Dimethyl-pyridinium chloride, Formaldehyde oxime trimer
13	9.643	1.37	Carbonic acid, 2-chloroethyl 4-nitrophenyl ester, 6-Methoxybenzofuroxan
14	10.572	2.58	1,2,3-benzenetriol
15	10.884	1.78	Ethanol, 2,2′-sulfonylbis-1-propene, 1-methylthio-2-trifluromethyl-1,3,3,3-tetrafluoro-propylene glycol
16	11.909	1.34	4-Hepten-3-one, 4-methyl-benzenamine, 2-methoxy-5-[5-(1H-pyrazol-1-yl)-1H-1,2,3,4-tetrazol-1-yl]-2H-1,4-oxazino quinolone
19	17.956	0.56	[4,8-bis (decyloxy)-5-(4-flurobenzenzoyl) naphthalene, 1-(4-flurobenzoyl)-Vitexin
20	19.063	1.31	Tetradecanamide, Decanamide, 2,2-dimethyl-tetrahydro-[1,4] dioxo 6,7-diol
21	20.638	13.14	9-octadecenamide, (2-butanamide, 3,3-dimethyl-pentanamide)
22	20.823	1.38	Hexadecanamide, Tetradecanamide
23	23.349	6.62	Pentadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester, Hexadecanoic acid. Octadecanoic acid

that hydroxyl group corresponds to the inhibition mechanism. In fact, it forms hydrogen bonds with the polar side chains of the amino acids, thereby modifying the molecular conformation of enzyme along with its hydrophilic and hydrophobic characteristics, prompting a decline in enzyme activity.

In furtherance of the efforts to explore the anti-obesity potency of *P. granatum* fruits peel, we investigated pancreatic lipase activity, a key enzyme for lipid digestion and dietary fats absorption (Yang et al., 2014). Ethanolic extract of the peel was observed as the most potent inhibitor of pancreatic lipase. This *in vitro* anti-obesity activity of *P. granatum* fruits peel extract might be adduced to its phytoconstituents especially phenols and flavonoids. Previous studies have observed the presence of pancreatic lipase inhibitors in some natural sources (Inthongkaew et al., 2017; Hengpratom et al., 2018).

Angiotensin-converting enzyme is a key enzyme which is known to catalyze the conversion of angiotensin I into the active vasoconstrictor, angiotensin II. ACE inhibitors are one of the mechanistic therapeutic strategies targeted for stabilizing blood pressure in hypertensive patients. The anti-hypertensive activities of medicinal plants act through the inhibition of ACE. A wide range of medicinal plants with ACE inhibitory activities have been reported in the past (Barbosa-Filho et al., 2006), and this activity was attributed to the synergistic action of secondary metabolites viz. alkaloids, flavonoids, tannins, proanthocyanidins, fatty acids, and terpenoids (Loizzo et al., 2007; Park et al., 2017). The ACE inhibitory activities of extracts might be because of flavonoid, alkaloid, and tannin contents, possibly through sequestration of enzyme metal co-factor, protein precipitation or through other mechanisms.

FT-IR and GC-MS chromatograms of ethanolic extract of fruits peel revealed the occurrence of a diverse bioactive components, particularly 1,2,3,4-butanetetrol, Dimethyl sulfone, 9-octadecenamide, and Pentadecanoic acid. Findings hypothesized that these bioactive metabolites may prevent metabolic diseases by inhibiting carbohydrate digestion and stimulating the secretion of insulin from pancreatic beta-cells (Hanhineva et al., 2010; Mohamed, 2014). In addition, these metabolites present in the extract could also be promising agents for exhibiting ACE inhibitory traits.

5. Conclusions

The present study demonstrated that *P. granatum* fruits peel extract attenuated enzyme inhibition in DM, obesity, and hypertension. In addition, FT-IR and GC-MS analyses of ethanolic extract of fruits peel revealed the occurrence of diverse bioactive compounds. Further extensive studies are required to get a deeper insight of not only on *in silico* molecular docking mechanisms of the bioactive substances in the metabolic pathway of obesity and hypertension in DM but also pivotal therapeutic role of *P. granatum* fruits peel through *in vivo* studies.

Declaration of Competing Interest

The authors declared that they do not have any conflict in publishing this research article.

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