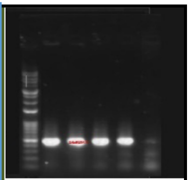
JOBIMB, 2023, Vol 11, No 2, 35-40
https://doi.org/10.54987/jobimb.v11i2.851

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



JOBIMB VOL 11 NO 2 2023
BlaNDM-1 MDR gene *A. baumannii*

Antioxidant and Antidiabetic Properties of Pectin Extracted from Pomegranate (*Punica granatum*) Peel

Hamsavartini M Segar[1], Shafinaz Abd Gani[1], Mohd Ezuan Khayat[1] and Mohd Badrin Hanizam Abdul Rahim[1,2]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia.
[2]Institut Biosains, NaturMeds, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Mohd Badrin Hanizam Abdul Rahim
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM,
Serdang,
Malaysia.
Email: badrin@upm.edu.my

HISTORY

Received: 7th Nov 2023
Received in revised form: 21st Dec 2023
Accepted: 30th Dec 2023

KEYWORDS

Pectin
Pomegranate peel
Antioxidant
Antidiabetic
Ultrasound-assisted extraction

ABSTRACT

Diabetes mellitus is a chronic disease resulting from defects in insulin secretion, insulin action or both. The prevalence of diabetes has been increasing gradually over the last few decades. It has emerged as a global health concern that burdens public health and socioeconomic development. Oxidative stress plays a crucial role in developing diabetic complications affecting the eyes, kidneys, nerves, and heart. There are various established adverse effects of current conventional antidiabetic drugs. Due to this fact, plants have been chosen for developing new drugs as the bioactive compounds in plants have higher antidiabetic and antioxidant properties with low toxicity. Pomegranate peel is an agro-industrial waste that is rich in pectin. Pectin is a natural polysaccharide that has gained importance due to its pharmacological properties. In this study, pectin from pomegranate peel was extracted using ultrasound-assisted extraction to characterize the pectin and to evaluate its antioxidant and antidiabetic activity. Pomegranate peel pectin (PPP) was extracted using citric acid (solid-to-liquid ratio of 1:30 g/mL) at pH 2 under optimized conditions at 75°C for 40 min. A white sponge-like solid was obtained upon freeze-drying with a yield of 18.17%. The moisture content of pectin was 18.26% and it was found to be a low-methoxylated pectin with a degree of esterification (DE) of 31.07%. Fourier Transform Infrared Spectroscopy (FTIR) results showed that pectin from pomegranate peel was quite similar to commercial pectin. The antioxidant activity was evaluated using scavenging assays, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 1 mg/mL (78.26% with IC_{50} value of 326.8 µg/mL, 79.12% with IC_{50} value of 83.63 µg/mL and 442.7 mg Fe^{2+}/g, respectively). The antidiabetic activity was assessed using an α-amylase inhibition assay at 1 mg/mL (43.82%). These results suggest that PPP has the potential to be used as an antidiabetic and antioxidant agents.

INTRODUCTION

Diabetes has emerged as a global health problem affecting public health and socioeconomic development. Although the prevalence of diabetes has begun to fall in some countries, it has risen in most emerging and developed countries in recent times. Diabetes mellitus develops when β-cells fail to generate enough insulin to maintain glucose homeostasis in the blood. Type 1 diabetes (T1D) and type 2 diabetes (T2D) mellitus are the two most common types of diabetes. If appropriate prevention strategies are not implemented, the figure will surge to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. T2DM treatments have enhanced glycaemic management and lower blood insulin levels [2]. Most of the diabetic patients have serious health problems due to diabetic complications. Both reactive oxygen species (ROS) and oxidative stress contribute to the development of diabetes and its complications [3]. These complications include cardiovascular disease, renal failure, neuropathy, visual impairment, and amputation [4]. Diabetes-related complications may be to blame for the higher morbidity, disability, and mortality rates among diabetics.

Various researchers and scientists have conducted extensive research to find the best treatment for this disease and its associated complications. Most of the antidiabetic agents have side effects and are unable to slow down the progression of diabetic complications. Drugs with low toxicity are in high demand because excessive use of chemical drugs can be detrimental to health. Medicinal plants are the major source of bioactive compounds with therapeutic benefits and for the discovery of new drugs. Many of the bioactive compounds are therapeutic in managing and treating chronic diseases like diabetes, obesity, cancer, and cardiovascular disease [5]. Foods such as legumes, beans, fruits, and plants contain various phytonutrients. Consumption of phenolic-rich fruits and vegetables has been associated with a lower risk of chronic disease development due to reduced oxidative stress and prevention of macromolecular oxidation [6].

Pomegranate, also known as *Punica granatum* is a drought-tolerant plant with a long life span [7]. Pomegranates can treat several diseases like high blood pressure, high cholesterol, oxidative stress, diabetes mellitus, and inflammatory activities [7]. Plant parts such as fruit peels, seeds, fruit shells and others are often treated as agro-industrial waste and contain active compounds that can help to cure various diseases. If these fruit wastes are not utilized properly, they can end up in landfills which will eventually lead to environmental issues. Pomegranate peel (PP) waste is abundant in pectin [8]. According to recent research, PP is a valuable waste with intriguing pharmacological activities due to its high concentration of phenolics, polysaccharides, and bio-transformed metabolites like urolithins [9]. Pectin is a natural polysaccharide that can be found in plant cell walls. It is widely used in food industries, cosmetics, and pharmaceutical industries. Thus, this study aimed to evaluate the antioxidant and antidiabetic properties of pectin extracted from the peel of *Punica granatum*, which could potentially be used in managing diabetes mellitus.

METHODOLOGY

Sample Preparation

Punica Granatum fruits were obtained from the local market. Then, the fruits were peeled and rinsed with distilled water to remove dirt. Then, the fruit peel was cut into smaller pieces of the same size.

Preparation of pectin

Drying of pomegranate fruit peel

The fruit peels were dried in an oven at 40°C overnight until a constant weight was achieved. Then, the dried fruit peel was ground into a powder using a dry blender before being stored in a desiccator at room temperature until further use.

Ultrasound-assisted extraction

The ultrasound-assisted extraction was conducted according to Chua et al. [10] with slight modifications. 60 g of pomegranate fruit peel powder was dissolved in 0.1 M citric acid at pH 2.0 and a solid-to-liquid ratio of 1:30 g/mL. The mixture was then placed in an ultrasonic bath at 75°C for 40 min. A constant power was maintained at 330 W and frequency at 37 kHz during the extraction process. The mixture was allowed to cool before filtering it through Whatman No. 1 filter paper. The filtrate was precipitated with 95% ethanol at a 2:1 (v/v) ethanol-to-filtrate ratio and then allowed to stand overnight (± 15 h) at room temperature (± 25°C). The precipitate was filtered and washed three times using ethanol 96%. The extracted pectin was dried in a freeze-dryer for three days. The equation below was used to calculate pectin yield:

Pectin yield (%) = (Weight of dried pectin (g))/ (Weight of dried powdered peels (g)) × 100%

Characterization of pectin

Moisture content

Moisture content was determined using moisture determination balance and it was done at the Institute of Tropical Forestry and Forest Product (INTROP), UPM.

Degree of esterification (DE)

The degree of esterification was performed according to Chua et al. [10]. 0.1 g dried pectin was dissolved in 10 mL distilled water after being wet with 1 mL ethanol. The mixture was swirled continuously to ensure thorough pectin dissolution. Then, 3 drops of phenolphthalein indicator were added to the mixture. The solution was titrated with 0.1 M NaOH and the volume (V1) needed to turn into a pale pink colour was recorded. 5 mL of 0.1 M NaOH was then added to the mixture. At room temperature, the mixture was stirred for 1 h. The mixture must be swirled with 5 mL of HCl until the pink colour fades and then another 3 drops of phenolphthalein were added to the mixture. The solution was titrated with 0.1 M NaOH, and the volume (V2) needed to turn into a pale pink colour was recorded. Using the equation below, calculate the degree of esterification:

$$DE\ (\%) = \frac{V2}{V1 + V2} \times 100$$

Where,

V1: initial titration volume
V2: final titration volume

Structural analysis

The functional groups of the pectin extracted from the pomegranate fruit peels were analysed using an FTIR Spectrometer Spectrum which was done at INTROP. The frequency range used was 4000-400 cm^{-1} [11]. It was then compared with the standard pectin.

Determination of antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

This assay was conducted based on the method of [12]. 20 µL of 1 mg/mL pectin was mixed with 180 µl of 0.1 mM DPPH in 100% methanol in a 96-well plate. Then, the plate was incubated for 30 min in a dark room. The absorbance reading was recorded using a microplate reader at 540 nm. Different concentrations of samples and ascorbic acid were prepared. The equation below was used to calculate the scavenging activity:

$$\text{Scavenging activity (\%)} = \frac{(Ac\text{-}As)}{Ac} \times 100\%$$

Where,

Ac = Absorbance of control
As = Absorbance of sample

Ferric Reducing Antioxidant Power (FRAP) assay

This assay was conducted based on [12]. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the volume ratio 10:1:1. Then, 20 µL of extracted pectin was mixed (1 mg/mL) with 180 µL of FRAP reagent in a 96-well plate. The plate was incubated for 30 min in a dark room.

The absorbance reading was recorded using a microplate reader at 593 nm. Different concentrations of ferrous sulphate ($FeSO_4.7H_2O$) as a standard and samples were prepared. The results were expressed as $FeSO_4$ equivalent mg/g extract.

2, 2-azinobis-(3-ethylbenzothazoline-6-sulphonic acids) (ABTS) assay

This assay was conducted based on [12]. The ABTS dye was prepared by mixing 5 mL of ABTS solution (7 mmol/L) with 88 µL of potassium persulfate solution (140 mM). Then, the mixture was incubated for 16 h in the dark at room temperature. The prepared ABTS solution was diluted with analytical grade ethanol to obtain an initial absorbance of 0.7 at 734 nm. 10 µL of the sample was added to a 96-well plate along with a 290 µL previously prepared ABTS solution. The mixture was incubated for 6 min in the dark room at room temperature. The absorbance reading was recorded using a microplate reader at 734 nm. Different concentrations of ascorbic acid as standard and samples were prepared. The equation below was used to calculate the scavenging activity:

$$\text{Scavenging activity (\%)} = \frac{(Ac-As)}{Ac} \times 100\%$$

Where,

Ac = Absorbance of control
As = Absorbance of sample

α-amylase Inhibitory Activity Assay

The inhibition assay was conducted according to [13] with slight modifications. The assay mixture contains 200 µL of 0.02 M sodium phosphate buffer pH 6.9 with addition of 0.006 M sodium chloride solution, 200 µL of α-amylase solution (2 U/mL) and 200 µL of 1 mg/mL of extracted pectin was incubated at 37°C for 15 min. Then, 200 µL of 1% starch solution was added as a substrate and re-incubated at 37°C for 15 min. The reaction was terminated by the addition of 200 µL of 3,5-dinitrosalicylic acid (DNS) reagent. It was then incubated in a boiling water bath at 90°C before adding 5 mL of distilled water. The absorbance reading measured the absorbance at 540 nm using a microplate reader. Negative control was prepared by replacing the sample with distilled water to show 100% enzyme activity, while a blank mixture was prepared without the enzymes. The inhibitory activity of the sample was compared with 1 mg/mL of acarbose as a positive control. The equation below was used to calculate inhibitory activity.

$$\text{Inhibitory activity (\%)} = \frac{A_{540}\text{ (negative control)} - A_{540}\text{ (sample)}}{A_{540}\text{ (negative control)}} \times 100\%$$

Statistical Analysis

All the experimental results were analysed and interpreted using GraphPad Prism software (Prism Version 8.0). All data values were expressed in triplicates as mean ± standard deviation. The overall statistical significance for each experiment at $p<0.05$ was assessed using an unpaired t-test.

RESULTS AND DISCUSSIONS

Extraction of pomegranate peel pectin (PPP)

Ultrasound-assisted extraction (UAE) method was applied in this present study to extract pectin from *Punica granatum*. Pectin extracted using conventional methods is not only low in quantity but also in quality, as extended exposure to heat during extraction can lead to the degradation of pectin [14].

The extraction was done at 70°C for 40 min to ensure promising extraction quality. [15] reported that temperatures ranging from 70 to 90°C can give higher pectin yield. Hence, 70°C was chosen since higher temperatures would cause the molecules of pectin composed of α-(1-4) linked units of galacturonic acid or methyl ester to degrade, leading to lower pectin yield. The highest pectin yield was 26.91% obtained at 45 min whereas the lowest pectin yield was 9.34% obtained at 30 min. It has been shown previously that raising the extraction temperature and time within a given range results in higher pectin yield, whereas decreasing or raising the temperature and time above a particular level results in low pectin production [16].

The extracted pectin was subjected to freeze-drying for further analysis. Freeze drying did not influence pectin content [17]. A sponge-like solid was obtained upon freeze drying and it dissolved very quickly in distilled water due to its soft texture. The texture appeared to be soft and can be crushed easily [18]. In this study, the pectin yield obtained was 18.17% under the optimized conditions. Previous studies reported that pectin yields were 6.8 to 10.1% [19], 23.87% [20] and 8.5% [21]. It can be deduced that pomegranate peels are a good source for pectin production.

Characterisation of pectin

The degree of esterification is commonly used to characterise pectin since it determines its gelling properties in industrial applications. Pectin is classified as low methoxyl pectin (DE >50%) or high methoxyl pectin (DE>50%) [10]. The titrimetric approach was used to determine the degree of esterification of pectin. In this study, the degree of esterification of pectin obtained from pomegranate peel was 31.07%. In agreement with this, several studies reported that the degree of esterification of pectin extracted from pomegranate peel ranged from 30-38% [11,19,22]. The pectin obtained from *Punica granatum* peel was categorised as LM pectin since the percentage DE was less than 50%. Low-methoxylated pectin can form gels with or without sugar in the presence of divalent cations. It is extensively utilised in the production of low-sugar foods such as jams and jellies [23].

Moisture content indicates the purity of pectin, and it was determined using moisture determination balance. The moisture content of extracted pectin was 18.26%. Pectin should have a low moisture content for safe storage and to inhibit microbial growth. Due to the formation of the pectinase enzyme, products with a high moisture content may lose quality [24]. In the previous studies, it was found that the moisture content of pomegranate peel pectin was 3.959% and 10.31%, respectively [25,26].

Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to confirm the functional group of pectin from pomegranate peel and standard pectin. An absorbance spectrum graph is produced by an FTIR spectrometer. It relies on infrared light to scan samples and examine bond properties. Various functional groups will absorb infrared radiation at different wavelengths. A catalogued spectra for known materials can be used to determine the unidentified sample. **Fig. 1** describes that extracted pectin and standard pectin had quite similar FTIR spectrums. Pectin is a polysaccharide that is in abundant carboxyl and hydroxyl functional groups [27]. The major peaks of hydroxyl groups appeared at 1735 cm^{-1} for pectin and standard pectin whereas the major peaks for carboxyl groups were shown at 3300 cm^{-1}.

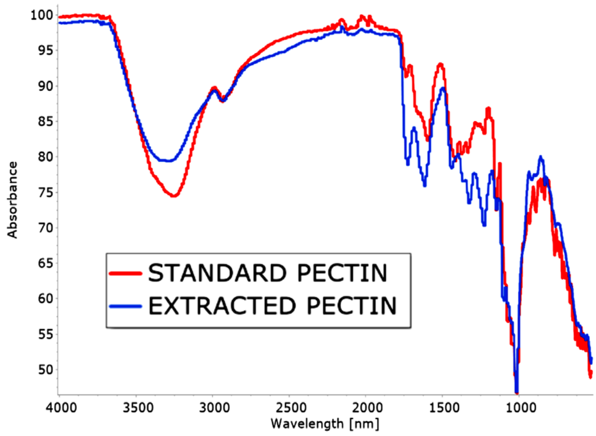


Fig. 1. FTIR spectra of standard pectin and extracted pectin.

Antioxidant activity

DPPH assay

The antioxidant activity of pomegranate peel pectin was measured by using DPPH, FRAP and ABTS assays. In the DPPH assay, the extract reduces a violet-coloured DPPH solution to a yellow-coloured product, diphenylpicryl hydrazine. It is extensively used as it can be done within a short period of time [28]. **Fig. 2** shows the scavenging activity of PPP with ascorbic acid as the standard. The scavenging activity of pectin at 1 mg/mL concentration was 78.26%.

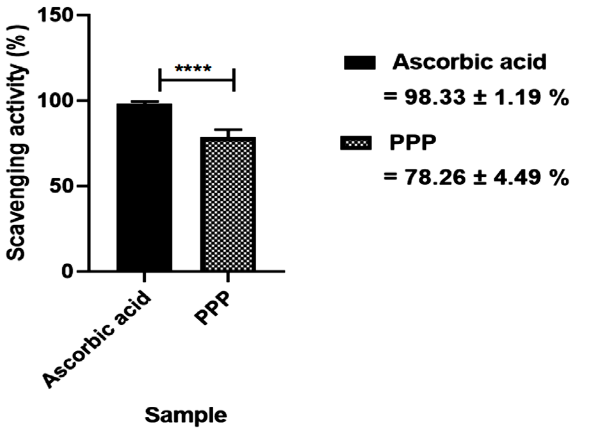


Fig. 2. The scavenging activity (%) of PPP and ascorbic acid as a positive control at a 1 mg/mL concentration. Data were expressed as mean ± SD (n = 3). **** indicates a high significant difference at $p < 0.05$ of unpaired t-test obtained from GraphPad Prism software. Error bars represent standard deviation.

The data are presented in IC_{50} values which represent the concentration needed to inhibit 50% of DPPH. **Fig. 3** shows the IC_{50} values of PPP and ascorbic acid acts as the positive control. Distilled water was used as the solvent. Based on the figure, ascorbic acid showed a significantly lower IC_{50} value of 149.5 ± 37.43 μg/mL compared to pectin.

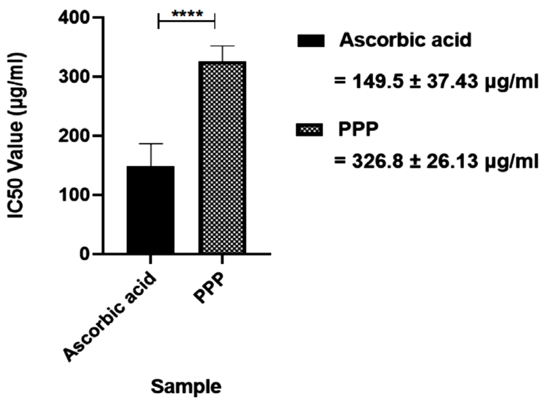


Fig. 3. The IC_{50} value (ug/mL) of PPP and ascorbic acid as a positive control for DPPH scavenging activity. Data were expressed as mean ± SD (n = 3). **** indicates a high significant difference at $p < 0.05$ of unpaired t-test obtained from GraphPad Prism software. Error bars represent standard deviation.

ABTS assay

The ABTS assay is regarded as one of the most sensitive assays to measure antioxidant activity because the antioxidant reaction has a faster reaction kinetics [29]. The ABTS is initially subjected to an oxidation reaction with potassium permanganate, potassium persulfate or 2, 2′-azo-bis (2-amidinopropane), producing the radical cation of the ABTS ($ABTS^{\bullet+}$) with a blue-greenish colour that absorbs at wavelengths of 415, 645, 734, and 815 nm. **Fig. 4** shows the scavenging activity of PPP with ascorbic acid as the standard. The scavenging activity of pectin was 79.12% at 1 mg/mL. This shows that the scavenging activity of pectin is in close proximity to the values obtained in DPPH and ABTS assay.

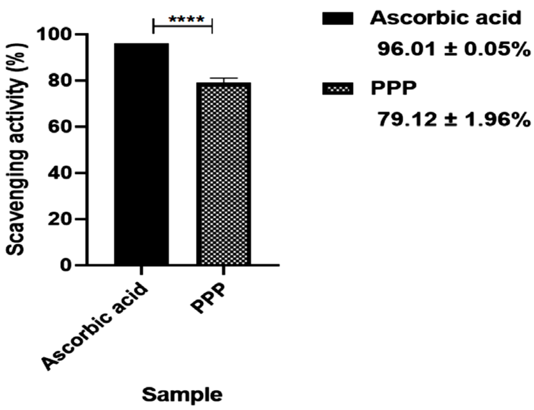


Fig. 4. The scavenging activity (%) of PPP and ascorbic acid as a positive control at the highest concentration (1 mg/mL). Data were expressed as mean ± SD (n = 3). **** indicates a high significant difference at $p < 0.05$ of unpaired t-test.

The data are presented in IC_{50} values which represent the concentration needed to inhibit 50% of DPPH. **Fig. 5** shows the IC_{50} values of PPP and ascorbic acid as the positive control. The IC_{50} value of pectin was 83.63 ± 26.02 μg/mL which is slightly higher than ascorbic acid. It was suggested that the presence of hydroxyl groups in polysaccharides such as pectin might be responsible for its high antioxidant activity [30].

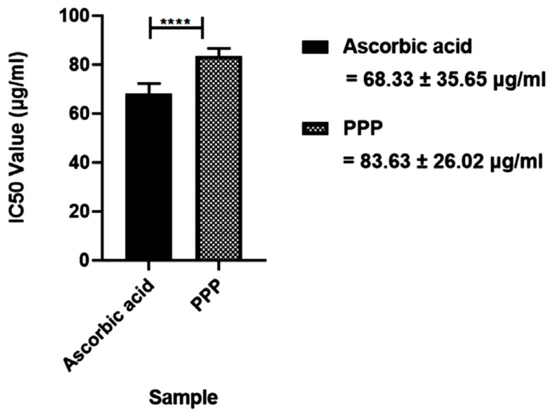


Fig. 5. The IC_{50} value (ug/mL) of PPP and ascorbic acid as a positive control for DPPH scavenging activity. Data were expressed as mean ± SD (n = 3). ** indicates a significant difference at $p < 0.05$ of unpaired t-test obtained from GraphPad Prism software. Error bars represent standard deviation.

FRAP assay

FRAP assay was also conducted to determine the antioxidant activity of pomegranate peel pectin. The antioxidant power is based on the reduction at low pH of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to an intense blue colour ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) with maximum absorption at 593 nm. **Fig. 6** shows the FRAP values of PPP with ferrous sulphate as the positive control. The FRAP values are presented as mg Fe^{2+}/g. PPP exhibited a lower FRAP value (442.7 ± 0.07 mg Fe^{2+}/g) compared to ferrous sulphate (706.2 ± 0.07 mg Fe^{2+}/g).

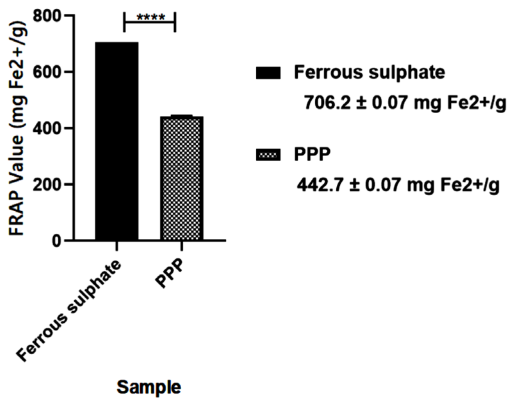


Fig. 6. The FRAP values of PPP and ascorbic acid as a positive control at a concentration of 1 mg/mL. Data were expressed as mean ± SD (n = 3). **** indicates a high significant difference at $p < 0.05$ of unpaired t-test obtained from GraphPad Prism software. Error bars represent standard deviation.

The Potential Effect of PPP in Inhibiting α-Amylase Activity

The antidiabetic activity of pectin from pomegranate peel was investigated by using α-amylase inhibitory activity assay. 3, 5-dinitrosalicylic acid (DNS) methods were carried out to determine the antidiabetic activity of pomegranate peel pectin. **Fig. 7** represents the inhibitory activity of α-amylase by PPP with acarbose as the positive control. The inhibitory activity of 1 mg/mL of extracted pectin was 60.61 ± 2.61% which is less potent than compared to acarbose (98.40 ± 0.95%). Acarbose is a well-known α-amylase inhibitor, hence the result is expected. Despite showing lower inhibitory activity than acarbose, PPP could still be considered as having an inhibitory effect of the enzyme. Previously studies reported that pectin extracted from passion fruit and citrus showed high antioxidant properties and a blood glucose normalizing effect on diabetic rats model [31,32].

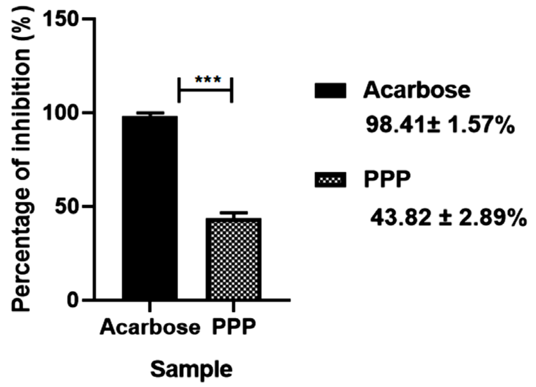


Fig. 7. The percentage of inhibition (%) of PPP and acarbose as a positive control at concentration of 1 mg/mL. Data were expressed as mean ± SD (n = 3). *** indicates a high significant difference at $p < 0.05$ of unpaired t-test obtained from GraphPad Prism software. Error bars represent standard deviation.

CONCLUSION

Our data demonstrated that pectin extracted from pomegranate peel fermented exhibits strong antioxidant activity in all three antioxidant assays. Moreover, the extracted pectin also had shown the potency to be the inhibitor of α-amylase. In short, PPP has the potential to act as an antioxidant and to serve as an antidiabetic agent for treating T2D. However, more studies need to be carried out to elucidate the pharmacological effects of PPP for the treatment of diabetes mellitus.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the Geran Putra, Universiti Putra Malaysia GP-IPM/2022/9717400 in funding this project.

FUNDING

This study was funded by the Geran Putra, Universiti Putra Malaysia GP-IPM/2022/9717400.

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019 Nov;157:107843.
2. Westman EC. Type 2 Diabetes Mellitus: A pathophysiologic perspective. Front Nutr. 2021 Aug 10;8:707371.
3. Kaludercic N, Di Lisa F. Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. Front Cardiovasc Med. 2020;7:12.
4. Jiang X, Meng W, Li L, Meng Z, Wang D. Adjuvant therapy with mushroom polysaccharides for diabetic complications. Front Pharmacol. 2020;11:168.
5. Devalaraja S, Jain S, Yadav H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. Food Res Int. 2011;44(7):1856–65.
6. Wang Y, Xiang L, Wang C, Tang C, He X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. PloS One. 2013;8(7):e71144.

7. Zarfeshany A, Asgary S, Javanmard S. Potent health effects of pomegranate. Adv Biomed Res. 2014;3(1):100.
8. Zhuang H, Chu S, Wang P, Zhou B, Han L, Yu X, et al. Study on the Emulsifying Properties of Pomegranate Peel Pectin from Different Cultivation Areas. Molecules. 2019;24(9):1819.
9. Fahmy HA, Farag MA. Ongoing and potential novel trends of pomegranate fruit peel; a comprehensive review of its health benefits and future perspectives as nutraceutical. J Food Biochem. 2022;46(1):e14024.
10. Chua BL, Tang SF, Ali A, Chow YH. Optimisation of pectin production from dragon fruit peels waste: drying, extraction and characterisation studies. SN Appl Sci. 2020;2(4):621.
11. Sathish S, Gowthaman KA, Augustus H. Utilization of *Punica granatum* Peels for the Extraction of Pectin. Res J Pharm Technol. 2018;11(2):613.
12. Clarke G, Ting KN, Wiart C, Fry J. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the malaysian rainforest. Antioxid Basel Switz. 2013;2(1):1–10.
13. Wickramaratne MN, Punchihewa JC, Wickramaratne DBM. In-vitro alpha amylase inhibitory activity of the leaf extracts of Adenanthera pavonina. BMC Complement Altern Med. 2016;16(1):466.
14. Bagherian H, Zokaee Ashtiani F, Fouladitajar A, Mohtashamy M. Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit. Chem Eng Process Process Intensif. 2011;50(11–12):1237–43.
15. Pereira PHF, Oliveira TÍS, Rosa MF, Cavalcante FL, Moates GK, Wellner N, et al. Pectin extraction from pomegranate peels with citric acid. Int J Biol Macromol. 2016;88:373–9.
16. Abid M, Renard CMGC, Watrelot AA, Fendri I, Attia H, Ayadi MA. Yield and composition of pectin extracted from Tunisian pomegranate peel. Int J Biol Macromol. 2016 Dec;93(Pt A):186–94.
17. Xu X, Zhang L, Feng Y, Zhou C, Yagoub AEA, Wahia H, et al. Ultrasound freeze-thawing style pretreatment to improve the efficiency of the vacuum freeze-drying of okra (*Abelmoschus esculentus* (L.) Moench) and the quality characteristics of the dried product. Ultrason Sonochem. 2021;70:105300.
18. Pandit AP, Koyate KR, Kedar AS, Mute VM. Spongy wound dressing of pectin/carboxymethyl tamarind seed polysaccharide loaded with moxifloxacin beads for effective wound heal. Int J Biol Macromol. 2019;140:1106–15.
19. Abid M, Cheikhrouhou S, Renard CMGC, Bureau S, Cuvelier G, Attia H, et al. Characterization of pectins extracted from pomegranate peel and their gelling properties. Food Chem. 2017 Jan 15;215:318–25.
20. Moorthy IG, Maran JP, Surya SM@, Naganyashree S, Shivamathi CS. Response surface optimization of ultrasound assisted extraction of pectin from pomegranate peel. Int J Biol Macromol. 2015 ;72:1323–8.
21. Yang X, Nisar T, Hou Y, Gou X, Sun L, Guo Y. Pomegranate peel pectin can be used as an effective emulsifier. Food Hydrocoll. 2018;85:30–8.
22. Akbari-Adergani B, Zivari Shayesteh P, Pourahmad R. Evaluation of some functional properties of extracted pectin from pomegranate peel by microwave method. J Food Technol Nutr. 2021;22;18(Summer 2021):5–16.
23. Norazelina Sah Mohd Ismail, Nazaruddin Ramli, Norziah Mohd Hani, Zainudin Meon. Extraction and characterization of pectin from dragon fruit (*Hylocereus polyrhizus*) using various extraction conditions. Sains Malays. 2012;41(1):41–5.
24. Chang KC, Dhurandhar N, You X, Miyamoto A. Sunflower head residue pectin extraction as affected by physical conditions. J Food Sci. 1994;59(6):1207–10.
25. Sharifi A, Hamidi-Esfahani Z, Ahmadi Gavlighi H, Saberian H. Assisted ohmic heating extraction of pectin from pomegranate peel. Chem Eng Process - Process Intensif. 2022;172:108760.
26. Güzel M, Akpınar Ö. Valorisation of fruit by-products: Production characterization of pectins from fruit peels. Food Bioprod Process. 2019;115:126–33.
27. Li H, Zhou H, Zhang J, Fu X, Ying Z, Liu X. Proteinaceous α-amylase inhibitors: purification, detection methods, types and mechanisms. Int J Food Prop. 2021;24(1):277–90.
28. Rahman MdM, Islam MdB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Res Notes. 2015;8(1):621.
29. Chanput W, Krueyos N, Ritthiruangdej P. Anti-oxidative assays as markers for anti-inflammatory activity of flavonoids. Int Immunopharmacol. 2016;40:170–5.
30. Xiong B, Zhang W, Wu Z, Liu R, Yang C, Hui A, et al. Preparation, characterization, antioxidant and anti-inflammatory activities of acid-soluble pectin from okra (*Abelmoschus esculentus* L.). Int J Biol Macromol. 2021;181:824–34.
31. Lacerda-Miranda G, M. Soares V, A. Thole A, Cortez E, Carvalho L, E.R. Caetano C, et al. Effect of Passion Fruit (*Passiflora edulis* f. flavicarpa deg.) Peel Flour on the Prognosis of Acute Pancreatitis after Overnutrition During Lactation. Nat Prod J. 2016;6(3):203–9.
32. Liu Y, Dong M, Yang Z, Pan S. Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway. Int J Biol Macromol. 2016;89:484–8.
27. Li H, Zhou H, Zhang J, Fu X, Ying Z, Liu X. Proteinaceous α-amylase inhibitors: purification, detection methods, types and mechanisms. Int J Food Prop. 2021 Jan 1;24(1):277–90.
28. Rahman MdM, Islam MdB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes. 2015 Dec;8(1):621.
29. Chanput W, Krueyos N, Ritthiruangdej P. Anti-oxidative assays as markers for anti-inflammatory activity of flavonoids. Int Immunopharmacol. 2016 Nov;40:170–5.
30. Xiong B, Zhang W, Wu Z, Liu R, Yang C, Hui A, et al. Preparation, characterization, antioxidant and anti-inflammatory activities of acid-soluble pectin from okra (Abelmoschus esculentus L.). Int J Biol Macromol. 2021 Jun 30;181:824–34.
31. Lacerda-Miranda G, M. Soares V, A. Thole A, Cortez E, Carvalho L, E.R. Caetano C, et al. Effect of Passion Fruit (Passiflora edulis f. flavicarpa deg.) Peel Flour on the Prognosis of Acute Pancreatitis after Overnutrition During Lactation. Nat Prod J. 2016 Sep 1;6(3):203–9.
32. Liu Y, Dong M, Yang Z, Pan S. Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway. Int J Biol Macromol. 2016 Aug;89:484–8.