INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0169/2024/32–2–129–135
DOI: 10.17957/IJAB/15.2185
http://www.fspublishers.org

Full Length Article

Chitosan Nanoparticles to Enhanced the Antioxidant and Antidiabetic Activities from *Phaleria macrocarpa* Ultrasonic Extract

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Received 04 April 2024; Accepted 23 April 2024; Published 22 June 2024

Abstract

In this study, nanoparticles were synthesised from an ultrasonic extract of *Phaleria macrocarpa* (Scheff.) Boerl (PmB) fruit peel using ionic gelation methods. The nanoparticles were then analysed with a particle size analyzer (PSA), a scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). The PSA analysis yielded a particle diameter of 338 nm and a polydispersity index (PDI) of 0.344 in the B1 variation. SEM analysis reveals that the surface of the resultant nano chitosan is composed of fine fibres. The infrared spectra changes after the production of cross-links; there is a new absorption at roughly 1600 cm^{-1} induced by the main amino group, which is identical to chitosan. Aside from that, ammonium ions in chitosan form cross-links with the extract. The efficacy of chitosan nanoparticles as an antioxidant and inhibitor of alpha-glucosidase (aGe) activity was successfully determined. Antioxidant activity using the DPPH method resulted in an IC_{50} value of 73.35 ± 0.08 μg/mL in the strong category, while with the FRAP method the EC_{50} value was 1.90 ± 0.05 μg/mL in the very strong category. The ability to inhibit aGe activity is very active with an IC_{50} value of 0.74 ± 0.001 μg/mL. The antioxidant and antidiabetic efficacy of ultrasonic PmB extract nanoparticles has never been evaluated. With this discovery, PmB plants can be converted into beneficial nutraceutical and medicinal chemicals.

Keywords: Nanoparticles; Ionic gelation techniques; Antioxidant; Alpha-glucosidase

Introduction

Phaleria macrocarpa (Scheff.) Boerl (PmB) is a botanical species that is widely used in Indonesian traditional medicine for its purported anti-inflammatory, antioxidant, anti-cancer and anti-diabetic attributes among others (Irawan *et al*. 2022b). Prior studies have demonstrated that an extract derived from the fruit peel of PmB, which was synthesised using ultrasonic waves and a 70% ethanol solvent, exhibits good antioxidant activity and functions as a good aGe inhibitor (Irawan *et al*. 2022a).

There have been numerous ways for increasing a substance's bioavailability. These strategies include boosting intestinal absorption, introducing new delivery channels, reducing particle size, and utilising solid dispersions. Nanotechnology is the process of reducing materials to smaller particle sizes using physical, chemical, and biological processes (Debnath *et al.* 2018). Nanoparticles (NnP) are defined as particles with a minimum dimension of 100nm and typically exhibit characteristics that are absent in bulk particles of the same molecular composition (Auffan *et al*. 2009). One material that has been widely used to make NnP is chitosan (El-Naggar *et al*. 2022).

Chitosan (Cs), a cationic polysaccharide, is a type of biopolymer that forms a straight-lined chain. The production of this substance involves the partial deacetylation of chitin (Yin *et al.* 2017). Due to their biocompatibility and biodegradability, Cs and its derivatives have emerged as one of the most viable options for producing NnP from vast quantities of natural substances (Liu *et al.* 2008). One method that can be used to produce chitosan nanoparticles is ionic gelation (Yanat and Schroën 2021). Cross-linking polyelectrolytes with pairs of multivalent ions constitutes the ionic gelation process. The formation of these cross-linked bonds will result in the particles acquiring ever-improving mechanics (Hoang *et al.* 2022).

Cs-based NnP can carry active ingredients, like natural and pharmaceutical products, through a number of delivery methods, such as intravenously and orally (Hasanifard *et al*. 2017; Mohammed *et al.* 2017). In contemporary times,

To cite this paper: Irawan C, M Sukiman, Rosalina, ID Putri, L Rahmatia, R Enriyani, Ismail, AF Alminda, A Utami (2024). Chitosan nanoparticles to enhanced the antioxidant and antidiabetic activities from *Phaleria macrocarpa* ultrasonic extract. *Intl J Agric Biol* 32:129–135

CsNnP have garnered significant attention in the fields of biomedical engineering, nanomedicine, and the advancement of novel therapeutic drug delivery systems. These systems aim to enhance pharmacological toxicity, specificity and bioavailability while decreasing overall toxicity (Sharifi-Rad *et al*. 2021).

The aim of the research is to produce CsNnP from ultrasonic PmB extract, which will then be characterized to ensure that the nanoparticles form. The CsNnP were tested for antioxidant and antidiabetic properties. It is envisaged that its significant antioxidant activity and inhibition of aGe would make it an effective antidiabetic medication.

Materials and Methods

Material

LIPI identified the PmB plant specimen and assigned it the voucher number 638/IPH.1.02/II.8/V.

Simplicia extraction

Irawan's investigations utilising the UAE approach with modified temperature control and real-time temperature sensors are relevant to this methodology. A steel vessel was then filled with 1.5 kg of PmB fruit peel simplicia powder, which had been weighed. As much as 10 L of a 70% ethanol solution were subsequently added. In order to monitor the extraction temperature in real time and generate mobile phone-accessible data, the mélange underwent sonication utilising an ultrasonic wave-assisted extractor outfitted with an automatic temperature sensor. The extraction was conducted at ambient temperature for 30 min with an amplitude of 0.6 m (Irawan *et al*. 2022a). The extraction procedure was repeated three times.

Synthesis and characterization of chitosan nanoparticles

The ion gelation process was utilised to synthesise CsNnP, which is a modified version of Putri et al.'s method. In the first step of the process, a total of 0.50, 0.75 and 1.00 g of Cs were weighed. Following this, a solution of 5% acetic acid (v/v) in 100 mL was used to dissolve the Cs and then a magnetic stirrer was used to homogenise the mixture. Therefore, a solution of 0.50, 0.75 and 1.0% Cs was produced. The next step was to make Na-TPP 0.50% by first weighing 0.50 g, then dissolving it with aquabides to a volume of 100 mL and finally homogenising it with a magnetic stirrer.

The Erlenmeyer was filled with 100 mL of Cs solution in 5% acetic acid with concentrations of 0.50% (B1), 0.75% (B2) and 1.00% (B3). Next, 1 mL of Tween-80 was added to each Cs solution and stirred at a speed of 1000 rpm for 10 min using a magnetic stirrer. Next, 0.1 gram of PmB fruit peel extract was added into each Erlenmeyer, and the mixture was stirred at 1400 rpm for 30 min with a magnetic stirrer. After adding 20 mL of 0.5% Na-TPP solution into B1, 30 mL into B2, and 40 mL into B3, stir again using a magnetic stirrer to stir the mixture at a speed of 1400 rpm for 2 h. The ultrasonic probe then sonicates the sample for 30 min. After the results are obtained, they are left for 24 h (Putri *et al*. 2018).

Fourier transform infrared spectroscopy (FTIR) is employed to characterise the resultant CsNnP through the identification and determination of functional groups. By employing a scanning electron microscope (SEM), the morphology of the CsNnP was ascertained, whereas a particle size analyzer (PSA) was utilised to quantify its size and distribution.

DPPH test for antioxidant activity

In order to evaluate the antioxidant capabilities of CsNnP samples, the DPPH test was utilised. This test is the same one that Irawan employed in his research (Irawan *et al*. 2022b). A micropipette was used to move 80, 160, 320 and 640 µL of the 1000 μg/mL CsNnP solution into a 5 mL volumetric flask that was lined with aluminium foil. It was mixed with methanol until the limit mark was reached, and then 1 mL of a 39 μg/mL DPPH solution was added. The concentrations of the solutions that were made were 16, 32, 64 and 128 μg/mL. For thirty min, the solution was stored in the dark at room temperature. The absorbance of the solution was measured using a visible spectrophotometer at 516 nm. BHT standards with concentrations of 2 to 8 μg/mL underwent the identical procedure. The antioxidant activity test for CsNnP and BHT was conducted three times.

A calculation was made using the following equation to determine the % inhibition value:

$$\%\ Inhibition = \frac{(A_{blank} - A_{sample})}{A_{blank}} x100\%$$

The regression line equation, which is Y = ax + b, will be created based on this equation. At the point where the percentage of inhibition is fifty percent, the IC_{50} value will be acquired.

FRAP test for antioxidant activity

In order to evaluate the antioxidant capabilities of CsNnP samples, the FRAP test was utilised. This test is the same one that Irawan employed in his research (Irawan *et al*. 2022a). A micropipette was used to move 5, 10 and 20 μL of the 1000 μg/mL CsNnP solution into a 5 mL volumetric flask. It was mixed with distilled water until the limit mark was reached, and then 0.4 mL of 0.001 *M* citric acid, 0.2 mL of 0.002 M Fe^{3+} and 0.4 mL of 0.2% o-phenanthroline were added. The concentrations of the solutions that were made were 1, 2 and 4 μg/mL. For 35 min, the solution was stored at room temperature. The absorbance of the solution was measured using a visible spectrophotometer at 510 nm. Gallic acid standards with concentrations of 1 to 6 μg/mL, underwent

the identical procedure. Each measurement of antioxidant activity on CsNnP and gallic acid was repeated 3 times.

A calculation was made using the following equation to determine the % reduction power value:

$$\%.\,Reduction\ Power = \frac{(A_{sample} - A_{blank})}{A_{sample}} x100\%$$

The regression line equation, which is Y = ax + b, will be created based on this equation. At the point where the percentage of reduction power is fifty percent, the EC_{50} value will be acquired.

Alpha-glucosidase inhibition test

The method used to determine aGe inhibitory activity refers to the Irawan procedure with slight modifications (Irawan *et al*. 2022a). Various concentrations of acarbose standard and CsNnP solution were made. Add 30 μL of acarbose solution and 17 μL of CsNnP solution to the p-Nitrophenyl-α-D-glucopyranoside solution using a micropipette. Incubation of the solution took place at 37°C for 5 min. Then, 17 μL of aGe solution was added and the temperature was maintained at 37°C for 15 min. After the incubation period, 100 μL of sodium carbonate at a concentration of 200 mM was added to the test solution in order to halt the reaction that was taking place between the enzyme and the substrate. The samples that served as controls had enzyme added after the addition of sodium bicarbonate solution. Using a microplate reader, the absorbance of the solution was measured at a wavelength of 405 nm. The aGe inhibitory activity test of each CsNnP and acarbose was carried out 3 times.

Results

Extraction yield

For the purpose of this investigation, ultrasonication was used to extract PmB fruit peel, and 70% ethanol was used as the solvent. A yield of 24.81% was achieved by extracting 372,1525 g of dry extract from 1.5 kg of the material that was used. In order to break down the cell walls of plants, ultrasonic waves are used.

Chitosan nanoparticles

A CsNnP suspension was prepared for this study. The suspension contained Cs with concentrations of 0.5, 0.75 and 1%, and the volumes of 0.5% TPP used were 20, 30 and 40 mL. PmB fruit peel produces CsNnP which forms colloids with a brownish colour (Fig. 1).

Characterization of CsNnP using PSA

The average particle size of the three CsNnP samples derived from PmB fruit peel extract synthesised with three different ratios of Cs to Na-TPP concentration was examined (Table 1 and Fig. 2). The diameter of the particles was found to be 338 nm in the B1 variation, which utilised a lower Cs to Na-TPP concentration ratio. CsNnP derived from PmB fruit peel extract satisfies the criteria for an NnP, as demonstrated by these finding.

Characterization of CsNnP using SEM

By employing a SEM, the morphology of the CsNnP was ascertained. Fig. 3 illustrates the morphology of CsNnP that was observed. The surface of CsNnP from an ethanol extract of PmB fruit peel is in the form of fine fibers, possibly derived from nano chitosan. In SEM, a stream of electrons is directed towards the sample being observed, then reflected by the sample surface and directed towards the detector to produce an enlarged and clarified image. The agglomeration is quite visible, requiring further drying of the sample.

Characterization of CsNnP using FTIR spectrophotometer

By utilising FTIR spectroscopy to characterise functional groups, the interaction between Cs with Na-TPP and CsNnP from an ethanol extract of PmB fruit peel was predicted. Variations in the wave number and intensity of each functional group indicate the interactions. Pure Cs exhibits a wide absorption band between 3200 and 3600 cm^{-1} in its infrared spectrum (Fig. 4). This band is caused by the stretching vibrations of the hydroxyl group (O-H) and amino group (N-H) that coincide, with the absorption at wave number 3326 cm^{-1}. Aside from that, an absorption at a wavenumber of 1636 cm^{-1} is observed in the fingerprint region; this is a characteristic absorption of primary amides. The infrared spectra showed a modification after crosslinking with Cs-NaTPP. The observed increase in absorption intensity of the O-H group in the 3300 cm^{-1} region is attributed to overlapping absorption with the N-H group. Following this, a distinct absorption occurs in the vicinity of 1600 cm^{-1}, which is attributed to the identical primary amide group as that of Cs (Fig. 5).

Antioxidant capacity using DPPH method

According to its ability to inhibit DPPH radicals, BHT has a higher IC_{50} value than CsNnP (Table 2). The IC_{50} value of CsNnP obtained was 73.35 ± 0.08 μg/mL which was categorized as a strong antioxidant, while the IC_{50} value of the acarbose standard gave an IC_{50} value of 4.52 ± 0.02 μg/mL. The increase in antioxidant activity is related to a 50% reduction in DPPH radical activity at doses equal to or less than the IC_{50} value.

Antioxidant capacity using frap method

The CsNnP concentrations used in this study were 1, 2 and 4 μg/mL. According to its ability to reduction Fe^{3+} to $Fe^{2+,}$

Table 1: Particle sizes of three variants of CsNnP from PmB fruit peel extract

Sample	Variation	Sample size		PDI
		Diameter (nm)	Wide (nm)	
CsNnP	B1	338	346	0.344
	B2	567	309	0.467
	B3	688	788	0.708

Information: B1 = 0.50% Cs; B2 = 0.75% Cs; and B3 = 1.00% Cs

Table 2: Antioxidant activity of CsNnP and BHT using the DPPH method

Sample	Linear regression equations	R^2 value	IC_{50} value (μg/mL)
BHT	y = 3.5989x + 33.732	0.9373	4.52 ± 0.02
CsNnP	y = 0.479x + 14.864	0.852	73.35 ± 0.08

Fig. 1: CsNnP synthesis outcomes from PmB fruit peel extract

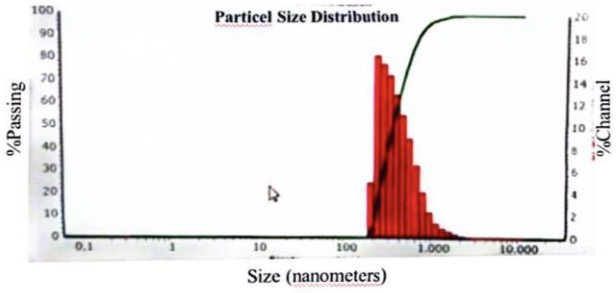

Fig. 2: Particle size distribution of CsNnP derived from PmB fruit peel extract

CsNnP has a higher EC_{50} value than gallic acid (Table 3). The EC_{50} value of CsNnP obtained was 1.90 ± 0.05 μg/mL which was categorized as a very strong antioxidant, while gallic acid as a standard compound had a higher EC_{50} value of 36.58 ± 0.06.

Alpha-glucosidase inhibitory activity

The CsNnP concentrations used in this study were 20, 40, 60 and 80 μg/mL. According to its ability to inhibit activity of aGe, CsNnP has lower IC_{50} value than acarbose (Table 4). The IC_{50} value of CsNnP obtained was 0.74 ± 0.001 μg/mL. The antidiabetic activity of CsNnP falls within the very active category, as indicated by its IC_{50} value less than 11 μg/mL.

Table 3: Antioxidant activity of CsNnP and gallic acid using the FRAP method

Sample	Linear regression equations	R^2 value	EC_{50} value (μg/mL)
Gallic Acid	y = 7.0639x + 28.236	0.9877	3.08 ± 0.05
CsNnP	y = 11.306x + 28.453	0.9818	1.90 ± 0.05

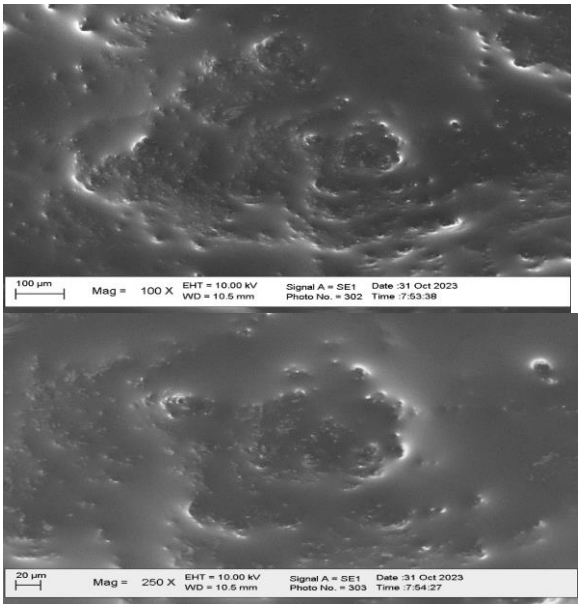

Fig. 3: SEM examination of CsNnP from an ethanol extract of PmB fruit peel

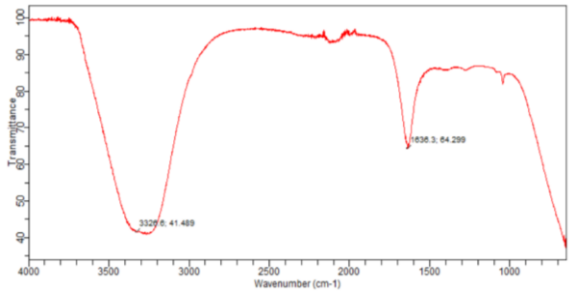

Fig. 4: Infrared spectra of pure Cs

Discussion

Ultrasonic extraction allows the solvents to penetrate deeper into the cells, which ultimately results in enhanced extract yields and improved process efficiency (Corrales *et al.* 2008; Garcia-Salas *et al.* 2010). It has been reported that the use of ultrasonic waves for the extraction of *Musa balbisiana* Colla results in the production of a substantial quantity of extract during a period of thirty minutes. This demonstrates that the utilisation of the UAE approach is both effective and cost-effective (Irawan *et al.* 2021; Putri *et al.* 2022).

The ion gelation process was utilised to synthesise CsNnP, which is a modified version of Putri *et al.*'s method

Table 4: The aGe inhibitory activity of CsNnP and acarbose as positive controls

Sample	Linear regression equations	R^2 value	IC_{50} value (μg/mL)
Acarbose	y = 1.0093x + 13.079	0.9882	36.58 ± 0.06
CsNnP	y = 22.972x + 32.844	0.9937	0.74 ± 0.001

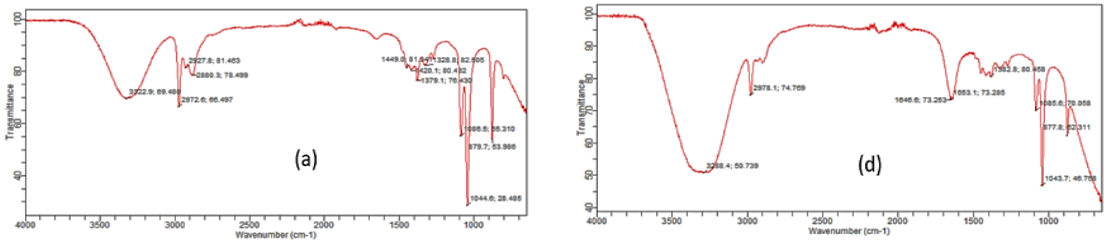

Fig. 5: The ethanol extract of PmB fruit peel IR spectra prior to (**a**) and subsequent to (**d**) crosslinking with Cs-NaTPP

(Putri *et al*. 2018). The interaction between Cs molecules and poly-anions resulted in the formation of intermolecular and intramolecular cross-links, which were facilitated by the poly-anions (Gan *et al.* 2005). Cs is a polycation polymer. Meanwhile, sodium tripolyphosphate (NaTPP) is a commonly used polyanion due to its effective crosslinking properties. Na-TPP will strengthen the mechanical strength of Cs because it has a negative charge with a high density, so that interactions with Cs polycations increase (Shu and Zhu 2002).

The utilisation of ultrasonics in the synthesis of CsNnP from an ethanol extract of PmB fruit peel is an additional factor that influences the NnP's size. The application of ultrasonic waves facilitates the physical reduction of NnP size. Ultrasonic waves initiate a strain-and-density cycle as they traverse a fluid. As a result of the negative pressure generated during stretching, the fluid molecules are attracted to one another and a vacuum is created; this vacuum subsequently generates bubbles that absorb the energy of the ultrasonic vibrations. Due to the energy absorption surpassing the energy discharge, the bubble undergoes expansion until it reaches a critical size, also known as the resonance size, which is contingent upon the fluid and sound frequency. When subjected to such conditions, the bubbles are unable to efficiently assimilate energy. Lack of input energy renders the bubble incapable of self-maintenance; as a result, it undergoes a violent explosion under the pressure of the surrounding fluid, which generates tremendous pressure. These extreme conditions disrupt chemical bonds, reducing the particle size (Hapsari 2009).

Utilising a PSA allowed for the quantification of its size and dispersion. If the average diameter of the synthesised particles is between one and one thousand nanometers, then the particles are considered to be of the CsNnP size (Mohanraj and Chen 2006; Buzea *et al*. 2007). In addition to the average particle size, PSA provides information regarding the Polydispersity Index (PDI) of CsNnP from an ethanol extract of PmB fruit peel. The PDI quantifies the degree of particle homogeneity. When the PDI value falls within the range of 0.1–0.7, it signifies that the resultant NnP are monodispersed, denoting a high degree of homogeneity. Conversely, NnP surpassing 0.7 in PDI value indicate the presence of a particle size distribution, which is characterised as broad or relatively less homogeneous (Abdassah 2017). Particle stability is proportional to size homogeneity; the greater the size homogeneity, the more stable the particle (Mardliyanti *et al*. 2012; Husniati and Oktarina 2014). .In this study, almost all nanomaterial variations showed good homogeneity except for the B3 variation of the CsNnP from an ethanol extract of PmB fruit peel. The sample is not homogeneous, which means the resulting nanoparticles have a wide size distribution. The B1 variation CsNnP sample, which possesses the smallest particle diameter (338 nm), underwent additional characterization by means of SEM and FTIR spectrophotometer.

The comparative infrared spectra of the ethanol extract of PmB fruit peel prior to and subsequent to crosslinking with Cs-NaTPP are illustrated in Fig. 5. Prior to the implementation of crosslinking or NnP formation, the extract demonstrated IR absorption results that were virtually identical. A low-intensity wide absorption of the O-H group is characteristic of alcohol compounds in the region surrounding 3300 cm^{-1}. Additional evidence in favour of the existence of an alcohol compound is the presence of intense absorption spectra in the 1000 – 1100 cm^{-1} region, which is characteristic of primary alcohols and pertains to the C-O bond. C-H sp3 absorption occurs in the region surrounding 2900 cm^{-1}, while aromatic C = C absorption occurs in the range of 1300–1400 cm^{-1} with modest intensity. So, the ethanol extract of the sample contains secondary metabolite compounds, as indicated by the data.

The infrared spectrum reveals a crosslink between the ammonium ion in Cs and the ethanol extract of PmB fruit peel, as the wave number shifts and the intensity increases. The hydroxyl groups present in the secondary metabolite compounds (suspected flavonoids) found in PmB fruit peel

extract are hypothesised to establish crosslinks with Cs. The N-H group in Cs can form a complex bond with the PmB fruit peel extract by reacting with the flavonoids' O-H group, which changes its charge to O^-.

The antioxidant activity of the crude extract of PmB fruit peel previously gave an EC_{50} value of 13.90 ± 0.11 μg/mL which falls into the very strong category. According to Fidrianny *et al*. (2015), the antioxidant activity of CsNnP falls within the strong category, as indicated by its EC_{50} value ranging from 50 to 100 μg/mL. Furthermore, the antioxidant activity test was carried out on the CsNnP which gave an EC_{50} value of 1.90 ± 0.05 μg/mL, which is also included in the very strong category. From the experimental results, there was an increase in the antioxidant activity of the CsNnP when compared to the crude extract. The antioxidant properties of CsNnP are ascribed to the hydroxyl groups of phenolic compounds that are utilised as crosslinkers and to the Cs content (Ojeda-Piedra *et al*. 2023).

Several studies have discovered a correlation between the ability of compounds that transfer protons, specifically phenolic compounds, to inhibit aGe activity. Phenolic compounds have been demonstrated to act as aGe inhibitors by competitively inhibiting carbohydrate digesting enzymes. This extends the process of converting carbs into glucose molecules (Patil *et al*. 2015; Jadalla *et al*. 2022; Omer *et al*. 2022).

Studies on the PmB fruit peel may assist in comprehending its biological properties, such as how it suppresses aGe activity and functions as an antioxidant. In the future, this comprehension may aid in the development of PmB plants as nutraceutical candidates and medicinal chemicals that benefit society.

Conclusion

In this study, nanoparticles were successfully synthesised from an ultrasonic extract of *Phaleria macrocarpa* (Scheff.) Boerl (PmB) fruit peel using ionic gelation procedures. The nanoparticles' characterization revealed the emergence of cross-links between ammonium ions in chitosan and sample extract. The antioxidant activity test on CsNnP using the DPPH method gave an IC_{50} value of 73.35 ± 0.08 μg/mL in the strong category, while the FRAP method gave an EC_{50} value of 1.90 ± 0.05 μg/mL in the very strong category. The ability to inhibit aGe activity is categorized as very active with an IC_{50} value of 0.74 ± 0.001 μg/mL. Future study should include testing antidiabetic efficacy *in vivo* and developing medication formulations.

Acknowledgements

The Ministry of Industry of the Republic of Indonesia provided financial support for this research, through the Industrial Human Resources Development Agency. Research instruments and laboratories also use the Politeknik AKA Bogor facilities.

Author Contributions

CI was responsible for the design of the experiments, the compilation of the findings, and the interpretation of the results; AU, RR, AFA and LR were responsible for the execution of the experiments, the compilation of the findings, and the interpretation of the results; MM, I, R, and IDP were responsible for the compilation of the findings, the statistical analysis of the results, the execution of the experiments, and the interpretation of the results.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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