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ANTIOXIDANT CAPACITY AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF CHITOSAN NANOPARTICLES SYNTHESIZED FROM *ANDROGRAPHIS PANICULATA* (Burm.f.) WALL LEAF EXTRACT

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Abstract. *Andrographis paniculata* (Burm.f.) Wall (ApBW) is a climbing shrub with a height of up to 2.5 meters and has potential as traditional medicine. The objective of this investigation is to asses antioxidant Alpha Glucosidase Inhibitory (AGI) properties with potential as an antidiabetic agent of chitosan nanoparticles (CtoNPl) from ApBW leaf extract by several variations. The Y1 (0.5% Cto) variation using Particle Size Analyzer (PSA) revealed a particle diameter of 510 nm and a PDI figure of 0.0478. After cross-linking, the wave number shifts and the intensity in infrared spectrum increases. In wave number 3300 cm^{-1}, the absorption intensity of the O-H group, apart from that, there is a new absorption that occurs at around 1600 cm^{-1}. Antioxidant activity was classified as "medium" with an Inhibition Concentration (IC_{50}) value of 145.38 ± 0.07 µg/mL as determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique. The Effective Concentration (EC_{50}) value for antioxidant activity was ascertained to be 11.90 ± 0.05 µg/mL by Ferric Reducing Antioxidant Power (FRAP) technique, thereby situating it within the "very strong" classification. The inhibitory effect on AGl activity has a significantly active IC_{50} value of 0.72 ± 0.001 µg/mL.

Keywords: *DPPH, FRAP, infrared, AGI, Andrographis paniculata.*

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1. Introduction

The plant *Andrographis paniculata* (Burm.f.) Wall (ApBW) was a member of the *Acanthaceae* family (Irawan *et al*., 2024). Research has demonstrated that the presence of andrographolide in leaves facilitates the inhibition of glucose absorption and stimulates insulin release through the inhibition of alpha-glucosidase (Subramanian *et al*., 2006). In previous studies, we found that the ultrasonically extracted ethanol extract

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of ApBW leaves produced high total phenolic content, very strong antioxidant activity and highly active AGl inhibition (Irawan *et al*., 2024). The plant *Andrographis paniculata* (Burm.f.) Wall (ApBW) contains flavonoids polymethoxyflavone (Niranjan *et al*., 2010; Sudarsono *et al*., 2006; Chao & Lin, 2010). Flavonoids polymethoxy flavone is reported to be efficacious as diuretic. Mechanism of polymethoxy flavone flavonoids Reportedly works as a diuretic with street inhibit co-transport and decrease reabsorption of sodium and potassium ions into the urine and mechanisms of increased natriuresis and kaliuresis. However, there is still a lack of published research using ApBW ultrasonic extract nanoparticles as antioxidants or inhibitors of AGS activity. Pharmaceutical compounds in the form of nanoparticles (NPl) have many benefits, including increased permeability and sustained targeting of target cells. In addition, changing its size to the nanometer scale causes an increase in its physical and chemical properties compared to its initial size (Sriningsih *et al*., 2002; Thama *et al*., 2008).

One of the fundamental components of nanotechnology is NPl; nanotechnology is a field of study concerned with nanoscales (Agarwal *et al*., 2018). In the field of science, nanotechnology necessitates processes involving the manipulation of matter in order to produce new structures, devices and materials. The manipulation of matter warrants particular emphasis due to its near-atomic scale, which dictates the quantity of particles necessary (Alharbi *et al*., 2023). Nanotechnology can be applied in many fields, such as agriculture, biotechnology, pharmaceuticals, medicine, electronics and the environment (Esquivel *et al*., 2015; Spirescu *et al*., 2021).

Solid colloids, known as NPl consist of very small molecules within the nanometer range (10-1000 nm). They possess distinctive physical and chemical characteristics by virtue of their diminutive dimensions and expansive surface area. Without the aid of a microscope, their various chemical and physical forms are imperceptible (Spirescu *et al*., 2021; Agarwal *et al*., 2018). The production of NPl can be accomplished through the utilisation of natural polymers like proteins and polysaccharides, as well as synthetic polymers such as polystyrene (Agarwal *et al*., 2018).

Segmental alkaline deacetylation of chitin produces chitosan (Cto), a cationic linear polysaccharide. Cto is composed of N-acetylgucosamine and glucosamine molecules (Chawla *et al*., 2014). Extensive research has been devoted to this polymer on account of its favourable physicochemical characteristic, including high biocompatibility, nontoxicity, biodegradability and excellent water solubility; it also has the capability of functioning as a drug delivery system (Dhillon *et al*., 2013). The remarkable characteristic of Cto make it a viable material for potential medical applications (Rampino *et al*., 2013).

Chitosan nanoparticles (CtoNPl) can be readily synthesized using the ionic gelation technique. Ionic gelation is a straightforward, convenient and controllable method for producing CtoPl; it does not require the use of hazardous compounds or organic solvents (Wu *et al*., 2017). The ionic gelation process involves cross-linking polyelectrolytes with multivalent ion pairs. The particles will acquire enhanced and improved mechanics due to the format ion of these cross-linked bonds. For example, chitosan is a polyelectrolyte that is capable of undergoing reactions with tripolyphosphate, which is a multivalent ion. Two liquid phases, one containing multivalent ions and the other containing Cto, combine to produce NPl (Hoang *et al*., 2022).

Therefore, this research aims to synthesize NPl from extracts of ApBW leaves by using Ultrasonic Assist Extraction (UAE). We carried out the synthesis using ionic gelation techniques. Apart from that, it is also to determine the antioxidant activity and AGl inhibitory activity of the CtoNPl obtained. There has been no previous research that has examined nanoparticles of ApBW leaves extract especially for antioxidant activity.

2. Material and Method

Material that used for this study was ApBW with voucher number B-104/II.6.2/IR.01.02/2/2023, the ApBW plant specimen was identified by BRIN. The process for this study was preparation and extraction simplicial, process of nanoparticle synthesis and assessment for antioxidant and AGI. Chemical reagents that used for this research was ethanol 70% (Merck), Cto (Merck), 1,1- diphenyl-2-picrylhydrazyl (DPPH) 39 mg/L (Sigma-Aldrich), Glacial acetic acid (Merck), Ferric Chloride Solution 20 mmol/L (Merck), o-phenantroline, (Sigma-Aldrich), citric acid solution 0,001 M, $FeCl_3.6H_2O$ (Merck), alpha glucosidase enzyme derived from Saccharomyces cerevisae.

2.1. Preparation and extraction of simplicia

This methodology is related to investigations carried out by Irawan using the *Ultrasonic Assist Extraction* approach with modified temperature control with real time temperature sensors. 1 kg of ApBW leaf simplicia powder was weighed, then put into a steel vessel. Next, 10 L of a 70% ethanol solution was added. The mixture was sonicated using an ultrasonic wave-assisted extractor equipped with an automatic temperature sensor so that the extraction temperature (was designed by our team research) could be observed in real time and the data could be accessed via mobile phone. Extraction was carried out for 30 minutes at room temperature with an amplitude of 0.6 m (Irawan *et al*., 2024).

2.2. The processes of chitosan nanoparticle synthesis and characterization

The process of CtoNPl synthesis was conducted via ion gelation, an approach that was modified from the method utilised by Putri et al. (2018). In the initial stage, as much as 0.50, 0.75 and 1.00 grammes of Cto were weighed, then dissolved using a 5% acetic acid solution (v/v) in 100 mL and homogenised using a magnetic stirrer to produce a 0.50, 0.75 and 1.0% Cto solution. Next, sodium tripolyphosphate (Na-TPP) 0.50% was made by weighing 0.50 grams, then dissolving it using aquabides to a volume of 100 mL, then homogenizing using a magnetic stirrer.

The Erlenmeyer was filled with a solution of Cto in 5% acetic acid with concentrations of 0.50% (Y1), 0.75% (Y2) and 1.00% (Y3). Next, 1 mL of Tween-80 was added to each Cto solution and stirred at a speed of 1000 rpm for 10 minutes using a magnetic stirrer. Next, added 0.1 gram of ApBW leaf extract to each Erlenmeyer and used a magnetic stirrer to stir the mixture at 1400 rpm for 30 minutes. After adding 20 mL of 0.5% Na-TPP solution into Y1, 30 mL into Y2 and 40 mL into Y3, the mixture was stirred at a speed of 1400 rpm for 120 minutes using a magnetic stirrer. An ultrasonic probe then sonicated the samples for 30 minutes. The results obtained are then stored for a period of 24 hours (Putri *et al*., 2018).

The characterization of the CtoNPl that is produced involves the identification and determination of functional groups through the use of Fourier transform infrared spectroscopy (FTIR). An electron scanning microscope (SEM) was employed to ascertain the morphology of the chitosan (Cto), whereas a particle size analyzer (PSA) was utilised to quantify its size and distribution.

2.3. Assessment of antioxidants using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

CtoNPl samples were assessed for antioxidant properties via the DPPH technique, which refers to the technique used by Irawan et al. (2021).

The CtoNPl solution with a concentration of 1000 µg/mL was transferred using a micro pipette of 125, 250, 500 and 1000 µL each into a 5 mL volumetric flask lined with aluminum foil. 1 mL of 39 µg/mL DPPH solution was added and dissolved using methanol to the limit mark, then homogenized. The solution concentrations obtained were 25, 50, 100 and 200 µg/mL. The solution is placed in a dark place and kept at room temperature for 30 minutes. After that, the absorbance of the solution was determined using a visible spectrophotometer *Shimadzu 1800* at 516 nm. This work was carried out three times. The same thing was also done for BHT standards with concentrations of 2, 4 and 8 µg/mL.

The following equation was utilised in order to achieve the calculation of the percent inhibition value:

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

This equation will lead to the construction of a regression line equation, Y = ax + b. The value of the IC_{50} will be attained when the percentage of inhibition is equal to fifty.

2.4. Assessment of antioxidants via FRAP technique

CtoNPl samples were assessed for antioxidant properties via the FRAP technique, which refers to the technique used by Irawan et al. (2024).

The CtoNPl solution with a concentration of 1000 µg/mL was transferred using a micro pipette of 10, 20, 40 and 80 µL each into a 5 mL volumetric flask. Next 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 and dissolved using distilled water to the limit mark 10 mL, then homogenized. The solution concentrations obtained were 2, 4, 8 and 16 µg/mL. The solution is kept at room temperature for 35 minutes. After that, the absorbance of the solution was determined using a visible spectrophotometer *Shimadzu 1800* at 510 nm. This work was carried out three times. The same thing was also done for gallic acid standards with concentrations of 1, 3 and 6 µg/mL.

The following equation was utilised in order to achieve the calculation of the percent reduction power value:

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

This equation will lead to the construction of a regression line equation, Y = ax + b. The value of the EC_{50} will be attained when the percentage of reduction power is equal to fifty.

2.5. Assessment of alpha-glucosidase inhibition

The procedure, which Irawan et al. (2024) employed with minor modifications, is referred to as AGl inhibitory activity testing. A CtoNPl solution and acarbose standard were prepared in a variety of concentrations. CtoNPl solution was pipetted up to a volume of 17 µL and the acarbose solution was pipetted up to a volume of 30 µL using a micropipette. The mixture was subsequently added to the para-nitrophenyl-α-D-glucopyranoside substrate at a concentration of 4 mM. At a temperature of 37 Celsius degree, the solution was incubated for 5 minutes. This was followed by the introduction of 17 µL of AGl solution, which was incubated at 37 Celsius degree for 15 minutes. After incubation, 100 µL of sodium carbonate with a 200 mM concentration were added to the test solution to terminate the reaction between the enzyme and the substrate. A sodium bicarbonate solution was incorporated into the test control prior to the enzyme's addition. A microplate reader was employed to ascertain the solution's absorbance at a wavelength of 405 nm.

3. Result and Discussion

3.1. Extraction of simplicia

70% ethanol was employed as a solvent in this investigation to extract ApBW leaves through ultrasonication. The dry extract yield was 8.89%, with 88.9105 g produced from 1 kg of sample. Ultrasound extraction is believed to enhance mass transfer by means of the capillary effect. This allows for an increased number of solvent molecules to enter the plant tissue. Cavitation pockets will develop on the plant cell wall as a consequence of the ultrasonic radiation. Upon a predetermined time, the cavitation droplets explode. Upon the demise of these cavitation bubbles, the openings within the cell wall may expand. The sonication-vulnerable nature of the glandular portion of plant cells will result in the diffusion of cavitation bubbles (Carpentieri *et al*., 2021; Marhamati *et al*., 2020; Mehta *et al*., 2022).

3.2. Chitosan nanoparticle synthesis and characterization

The CtoNPl suspension was formulated using the ionic gelation method. This method involves ionic interactions between the positive charges of the Cto and a negative charge, TPP. Both of polyelectrolyte solution was reacted for 2 hours with magnetic stirring and continued with sonication for 30 minutes to improve dispersion from NPl. For this study, a suspension of CtoNPl was produced. The suspension had 0.5%, 0.75% and 1% Cto concentrations and 20, 30 and 40 mL of TPP 0.5%. The CtoNPl of ApBW leaves was synthesized in the form of a colourless colloidal liquid, as shown in Figure 1.

Figure 1. Synthesis results of CtoNPl from ApBW leaf extract

The characterization of the CtoNPl was by using PSA, SEM and FTIR spectroscopy. A PSA was used to quantify its size and distribution. The results of testing with PSA on CtoNPl can be seen in Table 1.

Table 1. The results of characterization testing with PSA on CtoNPl from ApBW leaf extract

Sample	Variation	Sample Size		PDI
		Diameter (nm)	Wide (nm)	
CtoNPl	Y1	510	194	0.0478
	Y2	789	219	0.0776
	Y3	907	362	0.1780

Information: Y1 = 0.50% Cto; Y2 = 0.75% Cto and Y3 = 1.00% Cto

The test results in Table 1 show that the average particle size of the three CtoNPl samples from ApBW leaf extract obtained from synthesis with 3 variations in the ratio of Cto to Na-TPP concentration, the Y1 variation with a smaller Cto to Na-TPP concentration ratio shows the size smaller diameter, namely particle with a diameter of 510 nm nanoparticle. These results indicate that CtoNPl from ApBW leaf extract meets the requirements as a NPl.

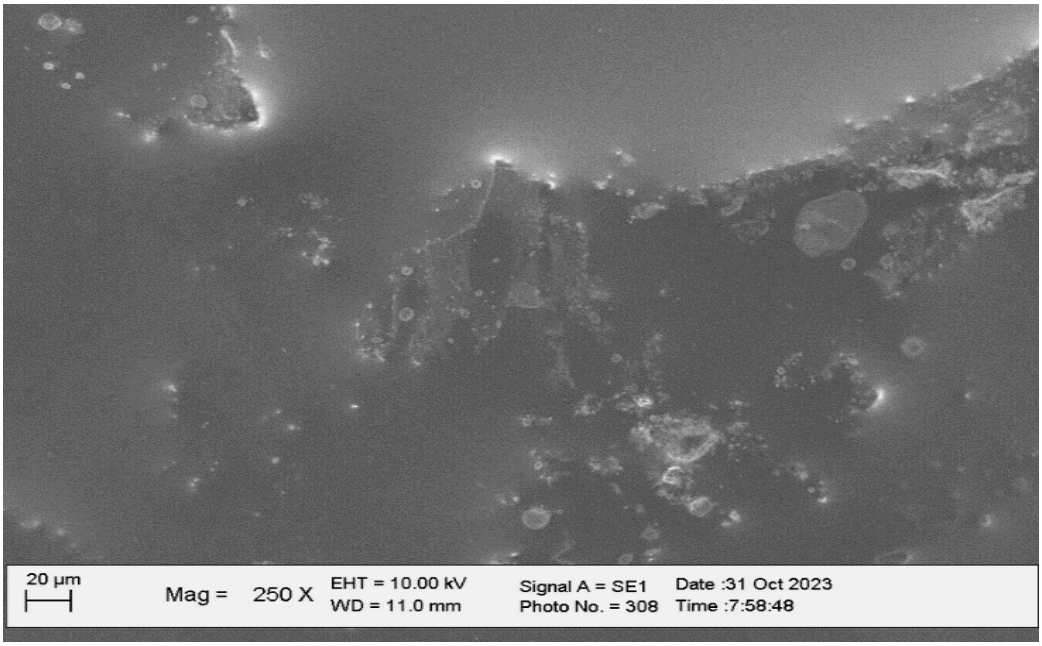
This result is better when compared with the average particle size formed with the addition of Cto and acetic acid. It has been reported that Cto in an acetic acid solution will produce an average particle size of 780-940 nm (Husniati *et al*., 2014). The comparison of the concentration ratio of Cto to Na-TPP in the CtoNPl synthesis influences this result. The particle size will increase as the concentration ratio of Cto to Na-TPP increases (Ayumi *et al*., 2018).

Ultrasonics are used to make CtoNPl from an ethanol extract of ApBW leaves, which is another thing that affects the size of the NPl. The use of ultrasonic waves helps to physically reduce the size of NPl. When ultrasonic waves travel through a fluid, a cycle of density and strain occurs. The negative pressure that occurs during stretching causes the molecules in the fluid to be attracted and form a vacuum which then forms bubbles which will absorb energy from the ultrasonic waves. As a result of the energy absorbed being greater than the energy released, the bubble expands to a critical size (resonant size) which depends on the fluid and sound frequency. Under these

conditions, the bubbles can no longer absorb energy efficiently. Without input energy, the bubble cannot maintain itself, the surrounding fluid will press on it and the bubble will experience a violent explosion, resulting in enormous pressure. These extreme conditions cause chemical bonds to break so that the particles become smaller (Hapsari, 2009).

In addition to determining the average particle size, PSA computes the Polydispersity Index (PDI) of CtoNPl from an ethanol extract of ApBW leaves. The particle distribution index (PDI) quantifies the particulate homogeneity. A high degree of homogeneity or effective monodispersity is indicated by a PDI value between 0.1 and 0.7 for the resultant NPl. In contrast, NPl with a size distribution that extends beyond 0.7 exhibit a wider range of sizes and are regarded as less uniform (Abdassah, 2017). According to Mardliyati et al. (2012), the level of particle stability is directly proportional to its size homogeneity. Particles of a smaller size are more stable. In this study, all variations of CtoNPl from an ethanol extract of ApBW leaves had monodisperse properties with good homogeneity. The size of the synthesised particles qualifies as NPl if they have an average diameter of 1-1000 nm (Buzea *et al*., 2007; Mohanraj *et al*., 2006).

Further characterization was carried out for the Y1 variation CtoNPl sample which has the smallest particle diameter (510 nm) using SEM and FTIR spectrophotometer. The morphology of CtoNPl was successfully observed as seen in Figure 2.



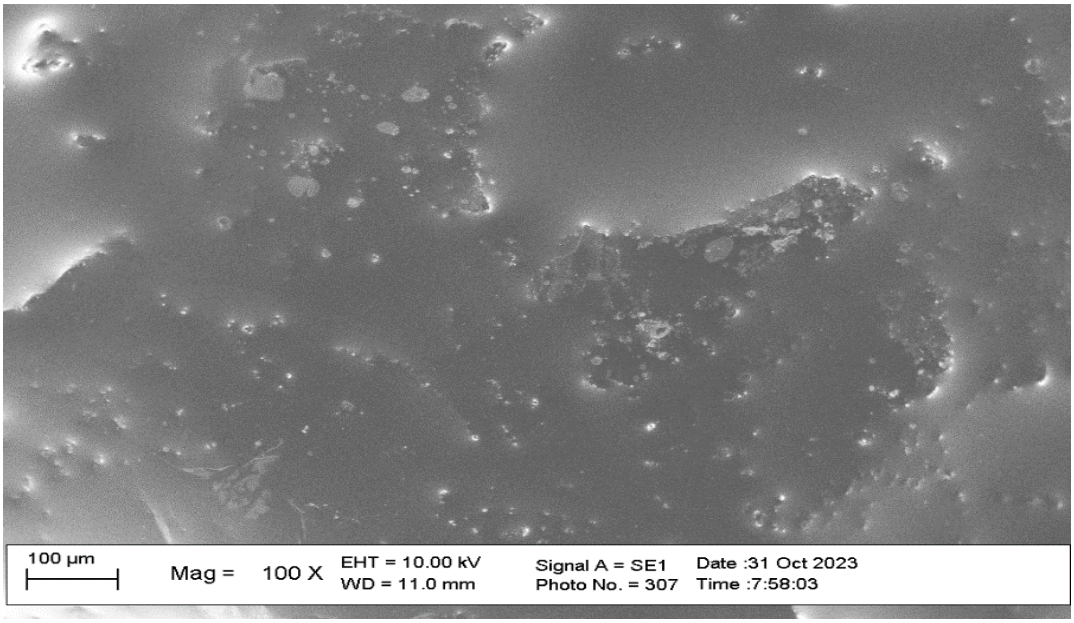


Figure 2. SEM analysis of CtoNPl from an ethanol extract of ApBW leaves

SEM was employed to ascertain the morphology of CtoNPl (Sun *et al*., 2022). This is important to do because it can influence the distribution process of the compound to the target tissue, cellular interactions and the level of toxicity of the compound (Frey *et al*., 2018). Based on Figure 2, fine fibers were visible on the surface of CtoNPl from an ethanol extract of ApBW leaves, but were hindered by large agglomeration of the sample.

Functional group characterization using FTIR spectroscopy was determined to predict the interaction between Cto with Na-TPP and CtoNPl from an ethanol extract of ApBW leaves. Shifts in the wave number and intensity of each functional group demonstrate the interactions. Based on Figure 3, the IR spectra results of pure Cto show a broad absorption band at wave numbers 3200 and 1636 cm^{-1}.

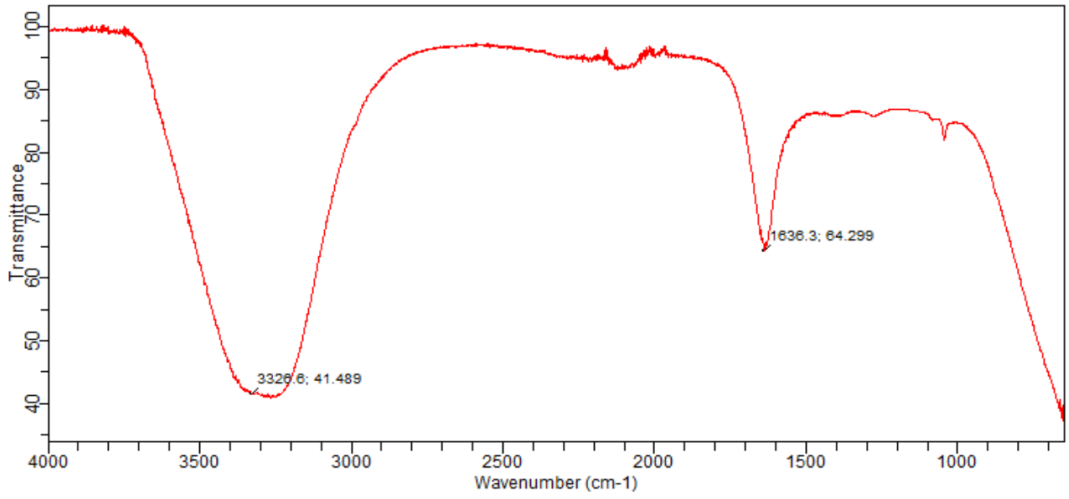


Figure 3. Pure Cto IR spectra results

Based on Figure 3, the IR spectra results of pure Cto show a broad absorption band at wave numbers 3200 to 3600 cm^{-1} due to overlapping stretching vibrations of the hydroxyl group (O-H) and amino group (N-H) with an absorption peak at a wave number of 3326 cm^{-1}. Apart from that, in the fingerprint area there is an absorption at a wave number of 1636 cm^{-1} which is a typical absorption of primary amides.

Figure 4 shows the comparative IR spectra results of the ethanol extract of ApBW leaves before and after crosslinking with Cto-NaTPP. Before carrying out crosslinking or NPl formation, the extract exhibited nearly identical IR absorption results on 3300, 1000-1100 and 1300-1400 cm^{-1}.

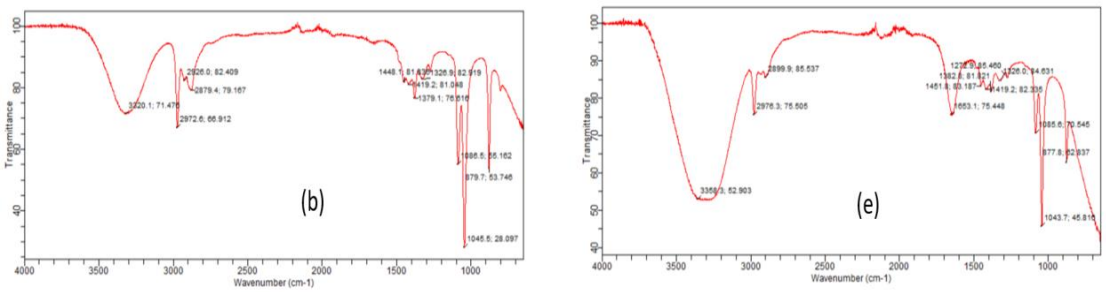


Figure 4. IR spectra results of the ethanol extract of ApBW leaves before (b) and after (e) crosslinking with Cto-NaTPP

After crosslinking with Cto-NaTPP, there was a change in the IR spectra results on the 3300 cm^{-1} area and there was an increase in the absorption intensity of the O-H group, which is believed to be due to overlapping with the N-H absorption. Then there is a new absorption in the area around 1600 cm^{-1} belonging to the same primary amide group as that of Cto (Figure 4). The IR spectrum shows that there is a crosslink between the ammonium ion in Cto and the ethanol extract of ApBW leaves because the wave number changes and the intensity goes up. Secondary metabolite compounds in ApBW leaf extract (suspected flavonoids) contain hydroxyl groups, these groups are thought to form crosslinks with Cto. The N-H group in Cto can interact with the O-H group in flavonoids which protonates to become O-, so that a complex bond is formed between Cto and ApBW leaf extract.

3.3. Assessment of antioxidants via the DPPH technique

The concentrations of CtoNPl made from the ethanol extract of ApBW leaves measured were 25, 50, 100 and 200 µg/mL, while the BHT concentrations used were 2, 4, 6 and 8 µg/mL. The assessment results are shown in Table 2.

Table 2. Data from the assessment of the antioxidant activity of CtoNPl samples using the DPPH technique

Sample	Linear Regression	R^2 Value	IC_{50} Value (µg/mL)
BHT	y = 3.5989x + 33.732	0.9373	4.52 ± 0.02
CtoNPl	y = 0.5122x + 12.666	0.8898	145.38 ± 0.07

Table 2 shows that the IC_{50} value of BHT based on its DPPH radical inhibition ability is higher compared to CtoNPl made from the ethanol extract of ApBW leaves.

The smaller the IC_{50} value, the greater the antioxidant activity, because the smaller the concentration required to reduce DPPH radical activity by 50% (Munteanu *et al*., 2021). The antioxidant activity of the CtoNPl made from the ethanol extract of ApBW leaves gave an IC_{50} value of 145.38 ± 0.07 μg/mL, when compared with the activity of the extract before it was made into CtoNPl which gave an IC_{50} of 327.52 ± 0.2 μg/mL, experiencing a quite significant increase in antioxidant activity.

The antioxidant activity of the CtoNPl is in the medium category because the IC_{50} value is in the range of 101-150 μg/mL, while the antioxidant activity of the crude ethanol extract of ApBW leaves is in the very weak category because the IC_{50} value is above 150 μg/mL (Fidrianny *et al*., 2015). Based on the antioxidant activity obtained from CtoNPl, it shows that the application of NPl technology to plant ethanol extracts through crosslinking between Cto-TPP can maintain its antioxidant activity through the mechanism of scavenging DPPH free radicals. It can be assumed that the increase in antioxidant activity against DPPH is due to the ability of NPl preparations to increase the stability of antioxidant compounds and increase the surface area of the extract, so that the contact between antioxidant compounds and free radicals is greater and more active in reducing the radical effect (Kavi *et al*., 2019; Piran *et al*., 2020).

3.4. Assessment of antioxidants via the FRAP technique

The concentrations of CtoNPl made from the ethanol extract of ApBW leaves measured were 2, 4, 8 and 16 μg/mL, while the gallic acid concentrations used were 1, 2, 3 and 4 μg/mL. The assessment results are shown in Table 3.

Table 3. Data from the assessment of the antioxidant activity of CtoNPl samples using the FRAP technique

Sample	Linear Regression	R^2 Value	EC_{50} Value (μg/mL)
Gallic Acid	y = 7.0639x + 28.236	0.9877	3.08 ± 0.05
CtoNPl	y = 4.3508x - 1.7956	0.9643	11.90 ± 0.05

Table 3 shows the EC_{50} value of CtoNPl of 11.90 ± 0.05 μg/mL while gallic acid as a positive control has an EC_{50} value of 3.08 ± 0.05 μg/mL. This result means that the antioxidant activity of gallic acid is relatively stronger than CtoNPl. However, CtoNPl, its reducing power is in the very strong category because it has an EC_{50} value of less than 50 μg/mL (Fidrianny *et al*., 2015).

Based on the information from the two antioxidant activity tests, it appears that CtoNPl made from the ethanol extract of ApBW leaves can act as an antioxidant through the DPPH radical scavenging mechanism and the oxidation-reduction mechanism. The antioxidant effect of these CtoNPl is attributed to the presence of hydroxyl groups of phenolic compounds incorporated as the crosslinker and Cto content (Ojeda-Piedra *et al*., 2023).

3.5. Assessment of AGl inhibition

The concentrations of CtoNPl made from the ethanol extract of ApBW leaves measured were 0.25; 0.50; 0.75 and 1 μg/mL, while the acarbose concentrations used were 10, 20, 30 and 40 μg/mL. The assessment results are shown in Table 4.

Table 4. Data from the assessment of the AGl inhibition of of CtoNPl

Sample	Linear Regression	R^2 Value	IC_{50} Value (μg/mL)
Acarbose	y = 1.0093x + 13.079	0.9882	36.58 ± 0.06
CtoNPl	y = 23.109x + 33.23	0.9926	0.72 ± 0.001

Table 4 shows the IC_{50} value of CtoNPl of 0.72 ± 0.001 μg/mL while acarbose as a positive control has an IC_{50} value of 36.58 ± 0.061 μg/mL. This result means that the AGl inhibition activity of CtoNPl is relatively more active than acarbose. However, CtoNPl, its antidiabetic is in the very active category because it has an IC_{50} value of less than 11 μg/mL (Lee *et al*., 2001).

Previous research has reported that the AGl inhibitory activity of the ethanol extract of ApBW leaves was 9.49 ± 0.04 μg/mL (Irawan *et al*., 2024). When compared between the extract and CtoNPl by converting it into NPl there was an increase in AGl inhibitory activity. The increased ability to inhibit AGl activity in CtoNPl results is in line with FTIR data information such as an increase in intensity in the O-H group at wave number 3300 cm^{-1} which overlaps with the N-H group, as well as the appearance of the amide group at 1600 cm^{-1}. Many studies have reported that the inhibitory properties of AGl activity in natural products are related to the presence of compounds that can donate protons, such as phenolic compounds. Phenolic compounds have been reported to act as AGl inhibitors by acting as competitive inhibitors of carbohydrate digesting enzymes, resulting in the hydrolysis of carbohydrates into glucose molecules taking longer (Jadalla *et al*., 2022; Omer *et al*., 2022; Patil *et al*., 2015). The presence of O-H and N-H groups in CtoNPl causes the formation of hydrogen bonds with the AGl receptor. Hydrogen bonds can occur in the -OH..O, -OH..N, -NH..O and NH..N groups. The receptor-ligand binding complex is stronger if it has hydrogen bonds. The more hydrogen bonds formed, the stronger the ligand-receptor bond and the Gibbs free energy value will be smaller (Rollando, 2018).

CtoNPl made from the ethanol extract of ApBW leaves extract inhibit the enzymes α-amilase pancreas working in hydrolyzing polysaccharide in caused by small intestine. CtoNPl made from the ethanol extract of ApBW leaves extract as inhibitor enzymes α-glucosidase also function in inhibits the expression of the glucose transporter SGLT1 and GLUT-4 translocation mRNA is increased by 3 times in diabetic patients who are permanently in the membrane apical resulting in an enzyme α-amylase and α-glucosidase also increased so that the taking of glucose in the intestine increased 3 times faster than the normal. This transporter functions to move glucose from one compartment to another compartment to pass through biological membranes so that glucose can be absorbed by the body (Doughni *et al*., 2012)

The findings of research conducted on ApBW leaf may enlighten us regarding its biological qualities, such as the manner in which it inhibits the activity of AGl and the manner in which it functions as an antioxidant. In the future, having such knowledge could make it easier to incorporate ApBW plants into the development of prospective nutraceuticals and therapeutic substances for the benefit of the community.

4. Conclusion

An investigation has been conducted into the bioactivity of chitosan nanoparticles synthesised from ApBW leaves extract, with a specific focus on the antioxidant and

AlGe inhibitor properties of the end product. Antioxidant activity was classified as "medium" with an IC_{50} value of 145.38 ± 0.07 μg/mL as determined by the DPPH technique. The EC_{50} value for antioxidant activity was ascertained to be 11.90 ± 0.05 μg/mL via the FRAP technique, thereby situating it within the "very strong" classification. The inhibitory effect on AGl activity has a significantly active IC_{50} value of 0.72 ± 0.001 μg/mL. The present study provides evidence of the effective fabrication and analysis of chitosan nanoparticles. Furthermore, by inhibiting AlGe, the resulting product is recognised for its exceptional potential as an anti-diabetic and antioxidant. Hence, chitosan nanoparticles derived from chitosan nanoparticles synthesised from ApBW leaves extract exhibit potential for advancement as nutraceuticals and can serve as a basic material for blood sugar regulation, thereby mitigating the likelihood of developing diabetes mellitus.

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