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Alginate-Based Nanoencapsulation on Ultrasonic-Assisted Extraction of *Parijoto* Fruit *(Medinilla Speciosa Blume)* and Its Antioxidant Activity

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Abstract

Parijoto (Medinilla speciosa Blume) has very strong antioxidant activity, but the bioavailability was low. Therefore, Parijoto should be formed into a nanoparticle. The research aimed to determine the characterization and IC_{50} value of nanoencapsulated Parijoto. Encapsulation was done using ionic gelation with alginate: $CaCl_2$ ratio 0.05%:0.05% (b/v). The ultrasonication was modified by a variation in frequency and sonication time. The characterization of nanoparticles was carried out using PSA to show the particle size and polydispersity index (pdI), and UV-Vis Spectrophotometer to show the percent of transmittance. The antioxidant activity was determined using FRAP assay. The characterization of pre-sonicated nanoextract was 265 nm of particle size, 0,472 of PdI, and 98,29% of transmittance. The best condition of sonication effect is given from 45 Hz of frequency and 15 minutes in time. The lower particle size from sonicated nanoextract was 218 nm, 0,415 of PdI, 99,56% percent of transmittance, and IC_{50} value obtained 1.696±0,014 *ppm with a very strong category.*

Keywords: *Alginate; antioxidant; parijoto; ultrasonication; ionic-gelation*

Introduction

The community has long used *Parijoto* fruit as an antibacterial, anti-thrush, and anti-inflammatory. It is also known that the *Parijoto* fruit extracts and fractions have extremely strong antioxidant activities (Vifta & Luhurningtyas, 2019). According to research by Vifta and Advistasari (2018) the *Parijoto* fruit's ethanol and ethyl acetate fractions both contain secondary metabolites of flavonoids, tannins, and saponins. The majority of pharmaceutical substances produced from natural ingredients have flaws, such as limited water solubility, a lack of permeability to cross the absorption barrier, and large particle sizes that reduce their bioavailability. Making nanoparticles is one strategy that can be used to solve this problem (Luhurningtyas *et al.*, 2020).

Nanoparticle technology offers various advantages in biomedical applications, especially for large particles to increase their surface area and magnetic properties (McNamara & Tofail, 2017). The main advantages of nanoparticles are the larger surface area, the faster beginning of the therapeutic effect, the higher solubility, and the higher oral bioavailability. Additionally, nanoparticle technology allows for the use of fewer doses, lower variability during feeding and fasting, and lower variability across patients (Ealias & Saravanakumar, 2017).

Ionic gelation is a method that can be used to produce polymer nanoparticles. The ionic gelation method utilizes the electrostatic interaction between the sodium alginate polymer and counterions in solution derived from encapsulated compounds or added cross-linkers such as Ca^{2+}. The concentration of the polymer and cross-linkers used affects the ability of the system to encapsulate proteins efficiently and determines the size of the particles formed and the compactness and surface stability of the resulting nanoparticles (Noore *et al.,* 2021).

Miranda (2016) reported that the use of sodium alginate at 0.3% w/v and $CaCl_2$ at 0.1% w/v with a volume ratio of 4:1 was able to produce nanoparticles that were stable in storage for seven days with a particle diameter of 739.8 nm and a zeta potential of -14.4 mV. Alginate nanoparticle characteristics, such as a readily adherent surface to the intestinal epithelium, a drug encapsulating process without the use of organic solvents, having high absorption qualities, and lack of toxicity, enable ideal drug delivery (Severino *et al.*, 2019; Nalini *et al.*, 2019). When used for drug delivery, polymer-based nanoparticles have extra advantages related to their biocompatibility and biodegradability properties (Chenthamara *et al.*, 2019).

It needs to be improved to produce a more homogeneous particle size distribution because previous research by Vifta and Luhurningtyas (2020) found that alginate-encapsulated *Parijoto* nanoparticles measuring 366.4 nm with a pdI of more than 0.5. Nanoparticles with a homogeneous size distribution will be more stable because the particles do not aggregate with each other, thus protecting the drug optimally and increasing its therapeutic ability and index (Noore *et al.*, 2021; Zielinska *et al.,* 2020). One effort that can be implemented is using the ultrasonication method. In this method, the formation of particles is influenced by the length of time of exposure and the frequency of ultrasonication (Rao *et al.*, 2021; Arya and Kumar, 2021).

This research was conducted to determine the characteristics of nanoparticles of *Parijoto* fruit extract encapsulated by alginate using ultrasonic waves, which included particle size, particle distribution as measured by the polydispersity index, percent transmittance, and antioxidant activity as seen from the IC_{50}. The research is expected to obtain results in increasing the activity of secondary metabolites from *Parijoto* fruit after being treated with time variations and ultrasonication frequency.

Research Method

Tools and Materials

The tools used are a rotary evaporator RE 2000-E, an OHAUS analytical balance, a magnetic stirrer Thermo Scientific Cimarec DHH-8, a magnetic stirrer Ika C-MAG HS 7, a set of Centrifuges PLC Series, an ultrasonic homogenizer UP100H, a spectrophotometer UV-Vis Shimadzu UV Mini 1240, a particle size analyzer (UV-1800 Shimadzu), a set of maceration tools, a set of glassware, and a waterbath.

The main material used is Parijoto fruit from Colo, Kudus Regency, Central Java with the specification of having red-purple color and had been determined at the Ecology and Biosystematics Laboratory of the Biology Department, Diponegoro University. The reagents used are ethanol 96%, pro-analytical ethanol, sodium alginate powder, $CaCl_2$ crystals, distilled water, aquabidest, $FeCl_3$ powder, NaOH crystals, KH_2PO_4 crystals, $K_3[Fe(CN)_6]$ powder, TCA crystals, oxalic acid crystals, and ascorbic acid powder Merck (Germany).

Procedures

Parijoto fruit extraction

Simplicia of *Parijoto* fruit was macerated with ethanol 96% (1:10). Maceration was carried out for two days, followed by remaceration for one day. The macerate was evaporated using a rotary evaporator at 70°C, then evaporated using a waterbath at 70°C until a thick extract was obtained.

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Nanoparticle formulation and characterization of *Parijoto* fruit extract

As much as 100 mg of *Parijoto* fruit extract was dissolved in 35 mL of ethanol, and mixed with 15 mL of aquabides. Take 10 mL of liquid extract and add 50 mL of alginate stock solution with a concentration of 0.05% w/v. Then, it was stirred for 30 minutes, and $CaCl_2$ solution was added and stirred for 3 hours (the volume ratio of alginate and $CaCl_2$ is 4:1). The mixture was stirred for one hour to form a colloidal nanoextract of *Parijoto* fruit. Then, the nanoextracts were sonicated at various times of 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 75 minutes, at frequencies of 45Hz and 80Hz. The nanoextracts were further characterized using a UV-Vis spectrophotometer and a Particle Size Analyzer (PSA).

Determination of Antioxidant Activity using FRAP Method

As much as 50 mg of the extract was dissolved in 50 mL of ethanol to obtain a concentration of 1000 ppm. Then, each solution was pipetted as much as 10µl; 30 µl; 50 µl; 70 µl; 90 µl dan 110 µl, to obtain concentrations of 1, 3, 5, 7, 9, and 11 ppm. After that, 1 mL of 0.2 M phosphate buffer (pH 6.6) was added to each concentration solution, mixed with 1 mL of 1% potassium ferricyanide, and incubated at 50°C for twenty minutes. 1 mL of 10% TCA was added to the solution and centrifuged at 3000 rpm for ten minutes. In a 10 mL measuring flask, 1 mL of supernatant was taken, 0.5 mL of 0.1% FeCl3 was added, and distilled water was added up to the mark. The absorbance of each solution concentration was measured at a wavelength of 696 nm with a UV-Vis spectrophotometer.

Antioxidant activity is determined by the Inhibition Concentration of 50% (IC_{50}). The results of % reducing Fe^{3+} from each concentration were followed by a linear regression calculation (x,y) to obtain an IC_{50} value, where *x* was the concentration (µg/ml) and *y* was the percentage of activity (%). The formula $y = bx + a$ is used to calculate IC_{50} samples and comparators. The IC_{50} value was obtained from *x* after replacing *y* with 50.

$$\%\ reducing\ Fe^{3+} = 1 - \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

Where $A_{control}$ is absorbance of control and A_{sample} is absorbance of sample.

Research Results and Discussion

Yield and active compounds of Parijoto fruit extract

Maceration of *Parijoto* fruit was carried out using 96% ethanol as a solvent. Extraction produces a thick brown extract with a characteristic odor. The extraction results in Table 1 show that the resulting extract is 45.61 grams with a yield of 10.13%.

The solvent used to extract *Parijoto* fruit is effective because the yield of the produced extract exceeds 10%. Ethanol 96% solvent was chosen as the solvent according to research conducted by Astutik *et al.,* (2021) which used ethanol 96% solvent with higher yields compared to ethanol 70% solvent. Another study by Muhammad *et al.*, (2021) also stated that ethanol solvents produce a greater yield than other organic solvents.

Table 1. The yield of Parijoto Fruit Extract

Powder Weight (gram)	Extract weight (gram)	Extract yield (%)	Organoleptic Characteristics		
			Form	Color	Odor
450	45.6067	10.13	Thick	Brown	Typical

The results of the phytochemical screening in Table 2 show the presence of secondary metabolites of flavonoids, tannins, and saponins in *Parijoto* fruit extract, it is in line with research conducted by Vifta and Advistasari (2018). The resulting *Parijoto* fruit extract contains a water content by the quality of the extract, which is equal to 2.86%, and the resulting extract is free from ethanol solvent.

Table 2. Phytochemical screening of Parijoto fruit extract

Type of Tests	Results
Flavonoids	+
Saponins	+
Tannin	+
Ethanol-free	+
Water content	2.86%

Flavonoids are active compounds that are available in all plant parts and have a variety of bioactivities (Fu *et al.,* 2021). By neutralizing free radicals when they form, flavonoids also have the potential to prevent the buildup of reactive oxygen species (ROS). It makes them potential antioxidant compounds (Dias *et al.,* 2021). Tannins produced from plant extracts contain chemicals that can be used as diuretics to treat diarrhea and reduce ulceration of the stomach and duodenal tumor tissue. By snatching up free radicals, tannins may potentially have anti-diabetic, antimicrobial, and antioxidant activities (Saini *et al.,* 2022). Saponins were found to have anti-inflammatory and antimicrobial activity and are compounds that are widely used for therapeutic activity (Raji *et al.*, 2019; Saini *et al.*, 2022).

Nanoencapsulation and Alginate Characterization of *Parijoto* Fruit Extract

The making of *Parijoto* fruit nanoextracts with alginate encapsulants was carried out using the ionic gelation method with and without ultrasonic treatment. The ultrasonic treatment is expected to improve the nanoparticle characteristics of the *Parijoto* fruit extract. The characterization results of *Parijoto* fruit nanoextract without sonication treatment produced particles with a size of 265 nm, a polydispersity index of 0.472, and a percent transmittance of 98.29%.

Ultrasonic treatment with variations in frequency and time produced better particle characteristics than before sonication. The characterization results in Table 3 show that there are differences in particle size and distribution as well as the percent transmittance of nanoparticles based on the changes in sonication frequency and time. Acoustic cavitation produced during the sonication process will produce waves and sonochemical reactions that cause changes in cells and the formation of particle pores (Rao *et al.,* 2021). In addition, the ultrasonication method has advantages such as using room temperature and requiring minimal time and energy (Arya and Kumar, 2021; Rao *et al.*, 2021).

Table 3. Characteristic results of parijoto fruit nanoextract

Sample	Frequency (Hz)	Time (Minute)	Transmittance (%)		Particle Size (nm)		pdI	
			Before	After	Before	After	Before	After
1	45	15	98.29	99.56	265.0	218	0.472	0.415
2	45	30	98.43	99.61	215.2	210	0.422	0.349
3	45	45	97.80	99.61	225.0	208.7	0.408	0.384
4	45	60	97.84	99.62	215.8	205.9	0.422	0.408
5	45	75	98.47	99.53	239.8	237.5	0.451	0.404
6	80	15	98.47	99.85	293.5	244.1	0.487	0.467
7	80	30	98.66	99.94	267.8	244.1	0.439	0.329
8	80	45	96.99	99.92	233.5	214.9	0.392	0.361
9	80	60	99.84	99.79	234.4	212.9	0.487	0.416
10	80	75	99.61	99.88	211.8	209.5	0.444	0.422

Nanoencapsulation of *Parijoto* fruit extract after sonication treatment produced various particle sizes and distributions. Some factors that influence particle changes in sonication are frequency, sonication time, vibration, strength, type of sonication, and temperature (Rao *et al.,* 2021). The sonication frequency and time have a significant effect on changes in the nanoparticle characteristics of the *Parijoto* fruit extract.

Based on the characterization results in Table 3, sonication using a frequency of 45 Hz

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for 15 minutes produced the most optimal particles compared to other variations. It produced particles with a size of 218 nm, a polydispersity index of 0.415, and a percent transmittance of 99.56%. These results were obtained based on the comparison or difference between the particle size and distribution values before and after sonication. Polymers have a size range of 20-250 nm and produce nanoparticles (Chenthamara *et al.*, 2019). Zielinska *et al.,* (2020) also stated that the size of polymer nanoparticles ranged from 1-1000 nm, and the trapped active compounds could distribute into and on the polymer surface.

The combination of the right sonication frequency and time produces particles with a smaller size compared to those produced without sonication treatment. Most of the sonication frequencies used in the extraction of natural products range from 20-120 Hz (Kumar *et al.,* 2021; Zia *et al.*, 2022). High frequency and low power intensity will produce radical reactions in the extraction process, while low frequency and high power will produce strong shear and mechanical forces during the sonication process, which can affect the formation of extract particles (Kumar *et al.,* 2021). In addition, the effect of sonication time has also been extensively studied (Syed Jaapar *et al.,* 2017). The increase in sonication time will affect the breakdown of the particles, making them smaller and more evenly distributed. However, if the sonication time is too long, it can damage the structure and matrix of the active compounds of natural ingredients (Sengar *et al.*, 2020; Rao *et al.*, 2021).

Antioxidant Activity of *Parijoto* Fruit Nanoextract

The antioxidant activity of *Parijoto* fruit nanoparticles was carried out using the FRAP (Ferric Reducing Antioxidant Power) method with the principle of the ability of antioxidant compounds to reducing Fe^{3+} ions to Fe^{2+} (Vifta and Luhurningtyas, 2020; Sethi *et al.*, 2020). Determination of antioxidant activitiy using the FRAP method takes into account the possibility of reduction of antioxidant molecules at certain concentrations. Antioxidant molecules will change the iron complex into neutral iron, which is marked by a color change as an indicator of the transfer of hydrogen atoms at a lower pH (Raji *et al.,* 2019).

In antioxidants measurement using the FRAP method, the reducing ability depends on the ability of the active compounds to ionize molecules (Sethi *et al.,* 2020). A lower ionization potential will produce easier electron abstraction. In this case, it considers the energy mechanism of bond dissociation and ionization potential that can produce the best antioxidant molecules to counteract degenerative disorders caused by oxidative stress damage to cells (Raji *et al.,* 2019).

Antioxidant determination also uses vitamin C as a comparison control. The results of the antioxidant activity given in Table 4 show that vitamin C produces an IC_{50} value of 1.629±0.015 ppm with a very strong antioxidant category. On the other hand, the antioxidant activity of the *Parijoto* fruit nanoextract before and after sonication shown in Table 5 gives a different result (p-value <0,05). The IC_{50} nanoextract values before and after sonication were 1.810±0,028 ppm and 1.696±0.014 ppm, respectively, in the very strong category.

Table 4. Antioxidant Activity of Ascorbic Acid

Concentration (ppm)	Mean of Abs±SD	%reducing Fe^{3+}±SD	Mean of IC_{50}±SD(ppm)
1	0.224±0.001	46.254±0.35	
2	0.254±0.003	52.509±0.51	1.629±0.015
3	0.308±0.001	63.643±0.25	(very strong)
4	0.352±0.002	72.577±0.44	
5	0.455±0.001	94.520±0.11	

Notes: The test was carried out three times.

Table 5: Antioxidant activity of nanoextracts before and after sonication

Concentration (ppm)	Before Sonication			After Sonication		
	Mean of Abs±SD	%Reducing Fe^{3+}±SD	Mean of IC_{50}±SD (ppm)	Mean of Abs±SD	%Reducing Fe^{3+}±SD	Mean of IC_{50}±SD (ppm)
1	0.223±0.0005	46.158±0.09	1.810±0.028 (very strong)	0.220±0.0005	44.897±0.00	1.696±0.014 (very strong)
3	0.290±0.002	57.425±0.37		0.283±0.001	57.755±0.28	
5	0.345±0.001	68.316±0.24		0.341±0.001	69.591±0.16	
7	0.380±0.0005	75.247±0.09		0.375±0.0001	76.530±0.16	
9	0.441±0.001	85.487±0.161		0.420±0.0005	85.714±0.09	
11	0.478±0.0005	97.551±0.096		0.495±0.001	98.019±0.161	

Notes: the test was carried out three times.

Encapsulation of *Parijoto* fruit extract plays an important role in protecting bioactive compounds from enzymatic degradation (Perinelli *et al.*, 2020; Noore *et al.,* 2021). Encapsulation is also able to control the resulting particle size to be smaller on both the micro and nanoscales. The encapsulated active compounds are more stable and have better effectiveness in their pharmacological activities (Noore *et al.,* 2021; Zielinska *et al.,* 2020).

Table 6. Tukey's HSD test for antioxidant activity of ascorbic acid and Parijoto fruit nanoextract

Sample	p-value	Information	Conclusion
S-1	0.001	<0.05	Significantly different
S-2	0.001		
S-3	0.003		

Notes:
S-1: Ascorbic acid
S-2: Nanoextract before sonication
S-3: Nanoextract after sonication

The IC_{50} value differences of *Parijoto* fruit nanoextract before and after sonication indicated that ultrasonication could increase the antioxidant activity of the extract during the encapsulation process by reducing and controlling particle distribution. The synergy between modified nanoencapsulation and ultrasonication can increase the stability of the active compounds in *Parijoto* fruit. In addition, *Parijoto* contains flavonoid compounds, which are effective as antioxidants and will be more effevtive after ultrasonication effect.

Flavonoids contain hydroxyl groups that can be donated to free radical molecules. Then, it forms new radical molecules that are stable and unreactive (Vifta and Luhurningtyas, 2020). Nanoencapsulation is an appropriate technique to ensure that the biological activity of active compounds with natural ingredients is maintained until these compounds reach and carry out their functions on the target (Noore *et al.,* 2021).

Conclusions and suggestions

Conclusions

The formulations of *Parijoto* fruit nanoextract before and after sonication treatment produced clear and transparent colloids. The sonication treatment produced a characteristically smaller particle size with a particle size of 218 nm, a pdI of 0.415, and a transmittance percentage of 99.56%. The best antioxidant activity in reducing Fe^{3+} ions was obtained with an IC_{50} value of 1.696±0.014 ppm in the samples treated with sonication for 15 minutes at a frequency of 45 Hz.

Suggestions

It is necessary to conduct further research regarding the effectiveness of the nanoparticle formula of *Parijoto* fruit extract using alginate encapsulants on several pharmacological activities both *in vitro* and *in vivo.*

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