



ENCAPCULATION OF GYRINOPS VERSTEEGII WITH IONIC GELATION METHOD

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ABSTRACT

Gyrinops versteegii's leaves have attracted research interest and have beneficial therapeutic effects through the antioxidant mechanism because of their high flavonoid content. Photosensitivity and low bioavailability, fast metabolism are the main limitations for its use, which can be overcome through encapsulation of extracts (flavonoids) into nanoparticle-based chitosan-tripolyphosphate by ionic gelation method. This study aims to encapsulate gaharu leaf extract and assess the characterization of the extract nanoparticles formed. Gaharu leaf nanoparticle extract has a particle size of 312.7 nm with a zeta potential of -16.2 mV and a polydispersity index value of 0.502, amorphous (non-uniform) morphology, has a tendency to be spherical, and has an imperfectly spherical shape, has functional groups -OH, -NH, C=O, and a phosphate group. Overall gaharu leaf extract is able to be encapsulated in chitosan-tripolyphosphate

KEYWORDS: *Gyrinops versteegii*, ionic gelation, nanoparticle.

INTRODUCTION

Agarwood leaf (*Gyrinops versteegii*) has received much attention from researchers because of its potential as a traditional medicine through its antioxidant mechanism. Agarwood leaves (*Gyrinops versteegii*) contain various chemical compounds as evidenced by research conducted by Parwata et al. (2018) which the methanol extract of gaharu leaves contains secondary metabolites such as phenolic compounds, terpenoids and flavonoids besides that in a study conducted by Kusuma et al. (2021) in the aqueous extract and ethyl acetate fraction of gaharu leaves, dominantly contained flavonoids in the flavonol group which have strong antioxidant activity with an IC₅₀ value of 60 ppm. Flavonoids are also present in the n-hexane fraction of gaharu leaves, namely the flavanone group which has the potential to be an active source of antioxidant compounds (Devathi et al., 2021).

Flavonoids are a group of natural ingredients that have various biological activities, and are widely used as therapeutic agents to treat diseases, and play an important role in the pharmaceutical industry (Brodowska, 2017). Several studies have shown that flavonoids are known to exhibit strong antioxidant activity (Obefemi et al., 2017). Secondary metabolites, one of which is flavonoids, have low bioavailability in

conditions of large particle size, fast metabolism and are unstable to the effects of high light intensity so that they are easily oxidized, so one potential strategy that can be applied is encapsulation into nanoparticle preparations that are more easily dispersed and stable in water (Liu et al., 2019).

The ionic gelation method is one of the methods applied to encapsulate extracts into nanoparticles by cross-linking mechanisms between chitosan using polyanion salts such as NaTPP. Agarwood leaf extract can be negatively charged in water because the hydroxyl groups of flavonoid compounds can carry out ionic interactions with the positively charged amine groups of chitosan (Detsi et al., 2020) and the addition of NaTPP which functions as a crosslinking agent to stabilize the formed nanoparticles (Kafshgari et al., 2012). Based on the background above, the researcher wanted to conduct research on the preparation of extract nanoparticles using the ionic gelation method and characterize the results obtained.

MATERIALS AND METHODS

Research Materials and Equipment

The material used in this study are Agarwood leaves (*Gyrinops versteegii*) obtained from the Kelaci area, Marga District, Tabanan Regency. The chemicals used

are aqua DM, glacial acetic acid, chitosan, sodium tripolyphosphate (NaTPP), Sodium Hydroxide (NaOH) 10%, Sulfuric acid (H₂SO₄), Hydrochloric acid (HCl), and Magnesium powder.

The tools used in this study included a set of maceration tools, filter paper, spatula, magnetic stirrer, a set of centrifugation tools, *Press Dryer*, *Particle Size Analyzer* (PSA), *Scanning Electron Microscopy* (SEM), IR spectrophotometer, and a set of glassware.

Method

Sample extraction

Agarwood leaf powder with a water content of 7.56% as much as 800 grams was extracted by maceration using aqua DM solvent which was heated at 70°C - 80°C. Maceration was carried out four times which produced 8L of brownish-red filtrate. The filtrate is then evaporated using *press dryer*.

Preparation of agarwood leaf extract nanoparticles

The encapsulation process was carried out by making 50 mL of a 2% agarwood leaf extract solution in aqua dm and 0.5% chitosan dissolved in 1% glacial acetic acid until the volume became 100 mL. 1% NaTPP solution was added as much as 50 mL drop by drop, stirring using a magnetic stirrer at 3800 rpm for 120 minutes. The colloidal nanoparticles formed were separated by centrifugation for 15 minutes at 3000 rpm. The precipitated nanoparticles formed were dried in a refrigerator to obtain nanoparticle crystals. The formed nanoparticles were analyzed using FTIR, SEM, and PSA to determine the characteristics of the nanoencapsulants.

Phytochemical test

2 milliliters of each extract solution and nanoparticles were put in 3 different test tubes. The first test tube is added 3 drops of concentrated HCl and a little magnesium powder, the second test tube is added 3 drops

H₂SO₄ and heated for 15 minutes, and the third tube added 3 drops of 10% NaOH. The color change that occurred was observed.

RESULTS AND DISCUSSION

Simplicia extraction

The sample used in this study was agarwood leaves obtained from Klaci Marga Village, Tabanan. The plant determination process was carried out at the LIPI-UPT Center for Plant Conversion of the "Eka Karya" Bali Botanical Garden.

Gaharu leaf simplisia as much as 800 grams was macerated with aqua dm at 70-80°C. The aqua dm used is at a temperature of 70-80°C which aims to maximize the maceration process because the higher temperature of the solvent will ruined the plant tissue more quickly so that the bioactive compounds contained in it can be withdrawn by the solvent (Parwata et al., 2018). The macerate obtained was evaporate with a press dryer to remove the maceration solvent. The water extract obtained was 45.32 grams.

Phytochemical result

The results of the phytochemical test with 10% Sodium Hydroxide (NaOH) reagent, Bate smith-Metcalfe and Wilstater produced a specific color in the extract and nanoextract obtained where the gaharu leaf extract obtained a yellow color with 10% NaOH reagent, red with Bate smith-Metcalfe reagent and Wilstater reagent. In the agarwood leaf nanoextract, a yellow-orange color was obtained with 10% NaOH, with Bate smith-Metcalfe and Wilstater reagents in yellow color. From these results it can be seen that the intensity of the most obvious color change is produced by the agarwood leaf nanoextract so that it can be said to have a higher flavonoid content than the gaharu leaf extract. (Vilmakumar et.al., 2014).

Table 1: Results of phytochemical tests of gaharu leaf extract and nanoextract.

Sample	Color test			Explanation
	NaOH 10%	Bate smith-Metcalfe	Wilstater	
Extract	Yellow	Red	Red	++
Nanoextract	Yellow-orange	Yellow	Yellow	+++

Explanation: +++: Highest content of flavonoids (brightest color intensity)

++ : High content of flavonoids (clear color intensity)

Garwood leaf extract nanoparticles

Gaharu leaf nanoparticles were prepared using the ionic gelation method, namely polyelectrolyte complexation between positively charged chitosan and negatively charged tripolyphosphate. This method was chosen because the process is simpler, avoids the use of high temperatures and can be controlled easily (Kurniasari and Athun 2017). The bioactive substances contained in gaharu leaf extract are trapped in cross-encapsulated chitosan-tripolyphosphate nanoparticles. Chitosan is dissolved in dilute acid solution to obtain chitosan cations, namely amine groups, the positively charged

amine groups will cross-link with the negative groups of the NaTPP polyanion to form a complexation between the different charges which causes the resulting chitosan nanoparticles to be more stable. (Putri et al., 2018). The NaTPP polyanion is formed because it dissociates in water releasing sodium ions and tripolyphosphate ions which then react with NH₃⁺ from chitosan. NaTPP salt is used to function as a crosslinking agent, because this material tends to have non-toxic properties, so this additional ingredient has been approved by the United States Food and Drug Administration (Kafshgari et al., 2012). The addition of the NaTPP solution drop by drop

accompanied by stirring using a magnetic stirrer aims to prevent solidification from occurring so that the resulting nanoparticles do not form large lumps, besides that to obtain a constant stirring speed (Suryadi et al., 2021).

The nanoparticles obtained can be seen in Figure 1.

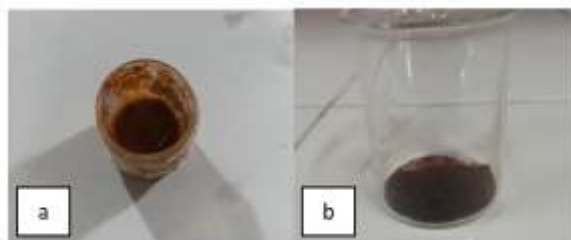


Figure 1: Agarwood leaf nanoparticles (a), agarwood leaf extract (b).

Characterization results with Particle Size Analyzer (PSA)

Particle Size Analyzer (PSA) used to see a formula that has become nanoparticles which can be known from the particle size distribution, zeta potential value and polydispersity index. The test was carried out 3 repetitions, the resulting particle size was 312.7 ± 3.09 nm.

According to Umair et al. (2016) sizes of 200 to 300 nm are considered suitable for crossing the biological barrier, avoiding RES (*Reticulo Endothelial System*), and avoid glomerular filtration. Sizes up to 400 nm are also considered acceptable (evaluated in the case of liposomes) (Alsavand et al., 2017). A study reported a suitable size for the effect of permeability and retention (EPR) which is in the range of 10–500 nm (Wu et al., 2017).

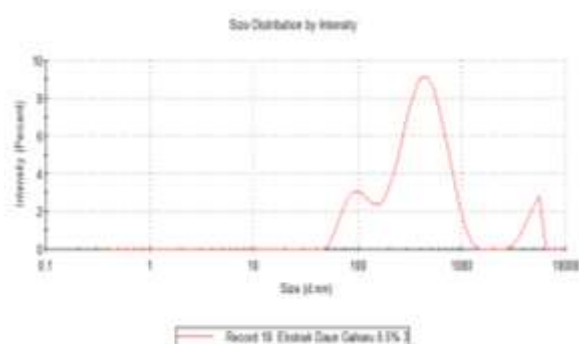


Figure 2: Particle size of agarwood leaf nanoextract.

The zeta potential value obtained from the gaharu leaf extract nanoparticles is -16.1 mv which provides short-term stability. As a rule of thumb, zeta potential values of ± 30 mV will provide good stability and ± 60 mV will provide very good stability.

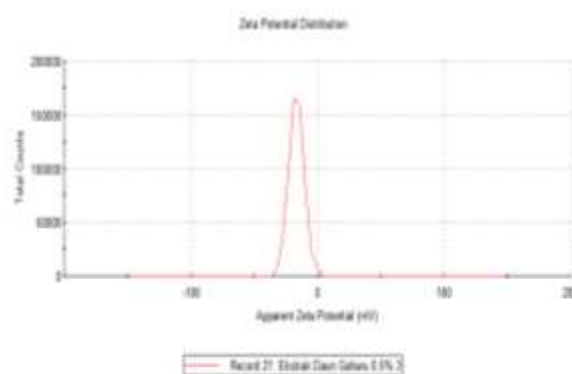


Figure 3: Zeta potential value of agarwood leaf nanoextract.

The average polydispersity index (PI) value obtained was 0.502 which indicated that the particle size was well controlled with a narrow dispersion. PI is a measure of particle homogeneity, ranging from 0 to 1, where the closer the PI value is to zero, the more homogeneous (mono-dispersed) the system becomes, while the higher the resulting PI value, the more unstable the formula will be.

Characterization results with Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a material characterization technique that is widely used to see the surface morphology of particles down to 1 nm in size. The results of SEM testing of agarwood leaf nanoextract can be seen in Figure 4.

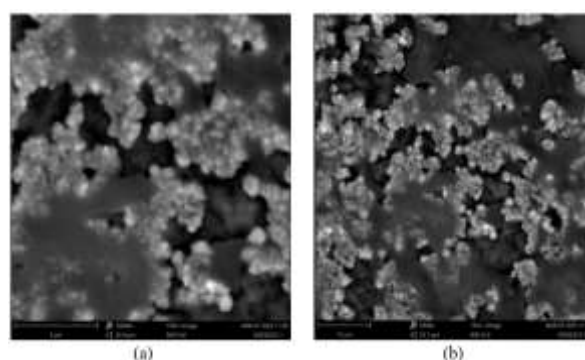


Figure 4: Results of Scanning Electron Microscopy of gaharu leaf nanoparticles with magnification 7500x(a), magnification 1000x(b).

The morphology of the agarwood leaf nanoextract shown in Figure 4 has a tendency to be spherical in shape, and there is an imperfectly round shape. Spherical nanoparticles have greater cellular absorption than rod shapes. The less spherical shape of the nanoparticles will facilitate contact between particles which will lead to aggregation (Oksal et al., 2020).

SEM tools are also equipped with EDX (Energy Dispersive X-Ray). EDX is a detector found in SEM tools. EDX is used to analyze the chemical composition present on the surface of the sample.

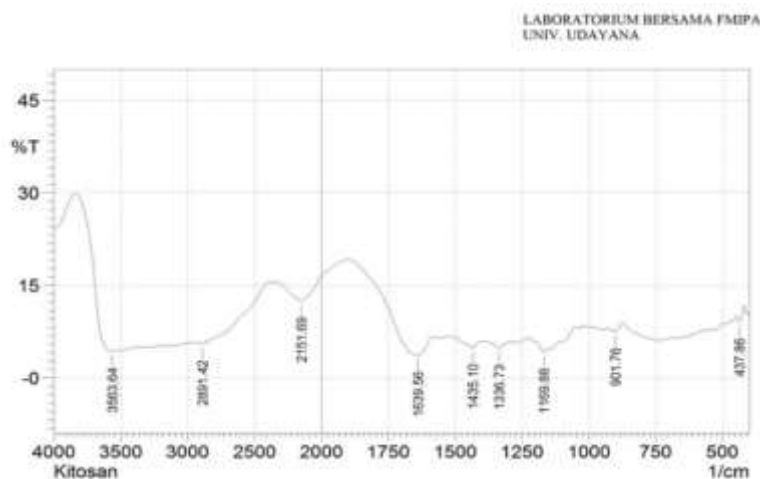
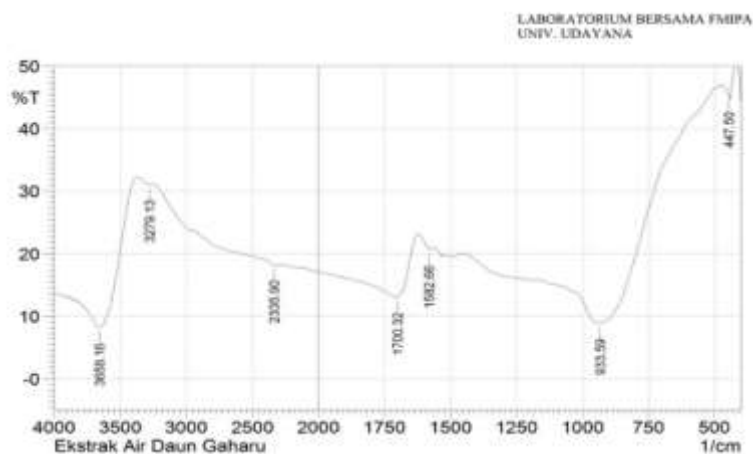
Table 2: Surface chemical composition of agarwood leaf extract nanoparticles using EDX.

Element Number	Element Symbol	Element Name	Atomic Conc. (%)	Weight conc. (%)
8	O	Oxygen	43.62	37.56
6	C	Carbon	24.53	15.86
11	Na	Sodium	18.15	22.45
7	N	Nitrogen	6.66	11.11
15	P	Phosphorus	3.46	6.45
19	K	Potassium	1.97	4.14
14	Si	Silicon	1.50	2.27
13	Al	Aluminium	0.11	0.16

The chemical composition based on the EDX in Table 2, is known to consist of 43.62% Oxygen (O), 24.53% carbon (C), 18.15% sodium (Na) and 6.66% nitrogen (N) as the main component. This indicates the formation of nanoparticles in the presence of chitosan biopolymer, NaTPP, and the active compounds contained in the ethylene acetate extract. Other elements found in small amounts such as P, K, Si, Al, while the element hydrogen (H) was not detected on the EDX. This is most likely because the hydrogen element is lighter than the other elements, so it is above the surface and cannot be detected by tools (Hakim, 2018).

Characterization results with *Fourier Transform Infra Red (FTIR)*

Characterization of nanoparticles agarwood leaf extract using FTIR serves to determine the change in functional groups indicating the occurrence of chemical interactions. The results of the spectra of chitosan, water extract and nanoparticles of agarwood leaf water extract with FTIR are shown in Fig. 5, Fig. 6 and Fig. 7, indicating that nanoparticles of agarwood leaf extract had been formed by the ionic gelation method.

**Figure 5: FTIR spectrum of chitosan.****Figure 6: FTIR spectrum of aqueous extract of agarwood leaves.**

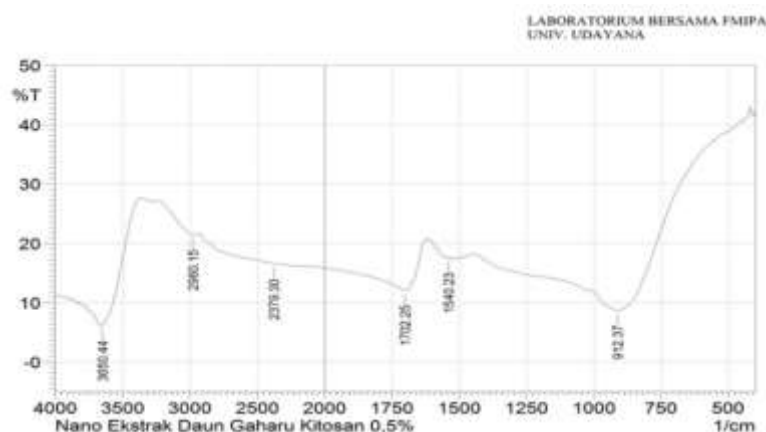


Figure 7: FTIR spectrum of agarwood leaf nanoextract.

Figure 5 shows the stretching vibration of the O-H groups in chitosan with wave number 3563 cm^{-1} and the stretching vibration of the N-H group appears at wave number 1639 cm^{-1} . In the FTIR results of nanoparticles of agarwood leaf extract, the wave number from N-H shifted to 1540 cm^{-1} . This shift in wave number is thought to be due to the interaction between chitosan and agarwood leaf extract, namely cross-linking between the NH_2 chitosan groups and Na-TPP and interactions with the -OH group. phenolics from flavonoid compounds in agarwood leaf extract (Putri et al., 2018). The discovery of a wave number of 912 cm^{-1} in the nanoparticles indicates the presence of a phosphate group which is a typical group of NaTPP (Oksal et al., 2020). In the spectrum of gaharu leaf extract the O-H group was detected at wave number 3658 cm^{-1} where in the spectrum of gaharu leaf nanoextract experienced a shift to 3650 cm^{-1} . This is due to the interaction between the -OH groups in chitosan and the -OH phenolic groups of flavonoid compounds in gaharu leaf extract (Putri et al., 2018). Absorption at wave number 1700 cm^{-1} with weak intensity indicates the presence of a C=O group. (Sastrohamidjojo, 2007).

CONCLUSION

Chitosan-tripolyphosphate-agarwood leaf extract nanoparticles were successfully prepared using the ionic gelation method with a particle size of $312.7 \pm 3.09\text{ nm}$. The color change of the phytochemical test results showed that the levels of flavonoids in the agarwood leaf nanoextract were higher than that of the gaharu leaf extract. Further research needs to be done to improve the stability of the agarwood leaf nanoextract produced by adding surfactants.

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