NANOPARTICLE FORMULATION OF LINDUR LEAVES (*Bruguiera Gymnorhiza*) USING CARBOXYMETHYL-CHITOSAN

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Abstract. Analgesic, antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, and anti-fungal advantages have been reported with a mangrove plant known as lindur (*Bruguria gymnorhiza*), which contains alkaloids, tannins, saponins, and steroids. The molecular size and solubility of this compound is very large. By the use of nanoparticle technology, it is possible to make medications more soluble by increasing the surface area of the particles. Carboxymethyl-chitosan (CMChi) polymer and CaCl2 are both used in the manufacturing of these nanoparticle. CMChi polymer concentrations of 200, 300, and 400 mg are used in the preparation of Lindur leaf extract nanoparticles, together with CaCl2 as a crosslinker. The ionic gelation-freeze drying technique was used to create nanoparticles. The obtained nanoparticles were evaluated for size and particle size distribution (PI), zeta potential, morphology and FTIR analysis. The smallest size and most uniform particle size distribution were formed by nanoparticles containing 200 mg of CMChi. The generated square nanoparticles are 196,5 ± 29,56 nm in size. Their PI values are 0,252 ± 0,053 and their zeta potentials are -17,43 ± 1.01 mV. According to the findings of the FT-IR study, the functional groups in the nanoparticles and the extract from *B. gymnorhiza* leaf are comparable.

Keywords: *B. gymnorrhiza*, carboxymethyl chitosan, nanoparticles, ionic gelation

1. Introduction

Indonesia is a country with a water area of 70% of the country's territory. This water area is covered with various types of mangrove plants which are reported to have potential as therapeutic agents for various diseases [1]. One of the plants that has been studied is Lindur (*Bruguria gymnorhiza*). Lindur leaves contain secondary metabolite compounds in the form of flavonoids, alkaloids, saponins, tannins, and steroids [2],[3],[4], which are reported to have analgesic, antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, insecticidal and anti-diabetic effects [5],[6],[7].

Dosage forms with poor delivery can reduce bioavailability and cause the drug to not produce optimal therapeutic effects. The World Health Organization in 2014 reported that 17,1% of the total existing essential medicines were classified as active substances in the BCS II (Biopharmaceutical Classification Scheme) class with good permeability but low solubility. In addition, 10,6% of them are classified in the BCS IV group (low permeability and low solubility). Drugs can be absorbed by the body and have pharmacological effects on the body if the drug is dissolved. Thus, the low solubility of the drug leads to low bioavailability [9],[10].

One drug delivery technology that can overcome this weakness is nanoparticles. Nanoparticles have a small size with a large surface area, so they can increase the solubility of compounds thereby increasing the pharmacological activity and bioavailability of drugs [11], [12]. One of the widely used nanoparticle formulation methods is ionic gelation. Ionic gelation

has the advantages of simple preparation, does not use dangerous organic solvents, and does not use heating which can damage the active substance [13],[14],[15].

Nanoparticles are particles with a size range from 1 nm to 1000 nm [14]. The development of nanoparticle technology requires the presence of polymers that function as drug carriers that can control drug release and have the ability to maintain drug stability. The polymers used can be synthetic and natural. Carboxymethyl chitosan (CMChi) is a derivative of chitosan which has good solubility in water. The amine and carboxyl groups contained in the molecule allow it to be used as a carrier in drug delivery systems. CMChi is biocompatible, biodegradable, and non-toxic. The mechanism for the formation of carboxymethyl chitosan nanoparticles using the ionic gelation method is based on the electrostatic interaction between the carboxyl group of carboxymethyl chitosan and the crosslinker of the $CaCl_2$ compound. The addition of $CaCl_2$ is intended to form a bond between the positive charge of the divalent cation Ca^{2+} ion in $CaCl_2$ and the negative COO^- ion from carboxymethyl chitosan [16].

2. Methods

2.1.Ethanol extraction of *B gymnorhiza* leaves

Simplicia *B.gymnorrhiz*a leaves were extracted by macerating them in a 96% ethanol solvent at a ratio of 500 mg of material to 2,5 liters of solvent until all of the samples were completely submerged in the solvent. Three 24-hour cycles of the maceration process are completed. Every 24 hours, a new solvent is added after it has been filtered using Whatman filter paper. When a thick extract was produced, the filtrate was further evaporated using a water bath and a Rotary evaporator[7].

2.2. Nanoparticle Formulation of Ethanol Extract of *B gymnorhiza* leaves

The composition of the *B gymnorhiza* ethanol extract nanoparticle formula is presented in the following table:

Table 1. Formulation of Nanoparticle of Ethanol Extract of *B gymnorhiza* leaves

Component	**Function**	**Formula % (b/v)**		
		F1	**F2**	**F3**
B. gymnorhiza extract	Active ingredient (mg)	40	40	40
Carboximethyl-Chitosan	Polymer (mg)	200	300	400
$CaCl_2$	Ionizing agent	100	100	100
Aquadest ad	solvent	100 mL	100 mL	100 mL

Carboxymethyl chitosan was dissolved in 100 mL of distilled water. Next, homogenization was carried out using a magnetic stirrer at a speed of 700 rpm for 1 hour. The second stage was making a 100 mg $CaCl_2$ solution which was dissolved in 40 mL of distilled water and added to the carboxymethyl chitosan solution then stirred until homogeneous using a magnetic stirrer at a speed of 700 rpm for 1 hour. The third stage of making the ethanolic extract of *B gymnorhiza* was weighed as much as 40 mg, then dissolved with 10 mL of 96% ethanol. In the fourth stage, the extract was mixed according to a predetermined formula and stirred using a magnetic stirrer at a speed of 600 rpm for 2 hours. After that, adjust it to pH 8 by adding 0,1 N NaOH solution.

2.3. Evaluation of Particle Size and Particle Size Distribution

Particle size measurements were carried out using a Particle Size Analyzer (PSA). The first step is that 5 µL of nanoparticles are diluted in 1 mL of distilled water and then analyzed using a Particle Size Analyzer (PSA). Then 1 mL of solution was placed into a cuvette and analyzed for particle size and size distribution.

2.4. Zeta Potential

Diluted ethanolic extract nanoparticles, and 1 mL of solution taken were placed into disposable folded capillary cells and analyzed for zeta potential using PSA.

2.5. Observation of Nanoparticle Morphology

Observation of nanoparticle morphology was carried out using a JSM 6510LA Scanning Electron Microscope (SEM). The first step is that the nanoparticles are dried using a freeze dryer. Then the nanoparticles are placed in the sample holder inserted into the holder and then analyzed [17].

2.6. FT-IR analysis

Chemical interactions and nanoparticle structure were observed using FT-IR Nicolet iS10. The first step is to make pellets by weighing potassium bromide (KBr) and then mixing it with nanoparticles. Next, the mixture is molded to form pellets. The next step is to carry out measurements using FT-IR in the infrared area with a wavelength of 7800-350 cm^{-1} [13].

3. Results And Discussion

The ethanol extract of *B.gymnorrhiza* leaves is made using the maceration method from 500 grams of *B.gymnorrhiza* leaf simplicia extract powder which is soaked in 2,5 liters of 96% ethanol and remacerated 2 x 24 hours. Then evaporate with a water bath. The extraction yield was 65,786 grams, blackish green in color, and the yield was 13,157%. The size of the extraction yield shows the effectiveness of the extraction process. The effectiveness of extraction is influenced by the type of solvent used as a filter, the size of the simplicial particles, the method, and the length of extraction [7].

Results of Nanoparticle Characteristics of *B. gymnorrhiza* Leaf Ethanol Extract.

3.1. Particle Size

Particle size evaluation was carried out on the three formulas with three replications. The particle size test results can be seen in the following table:

Table 2. Particle size of nanoparticles

Replication	Particle size (nm) ± SD (n=3)		
	F1	**F2**	**F3**
1.	217,4	296,5	306,2
2.	236,3	283,9	399,6
3.	175,6	277,9	351,5
Average	196,5	286,1	352,4
SD	29,56	9,49	38,1

The three formulas show the results for the size range of polymer nanoparticles that fall into the nanoparticle category, namely sizes in the range of 1-1000 nm [13],[18].

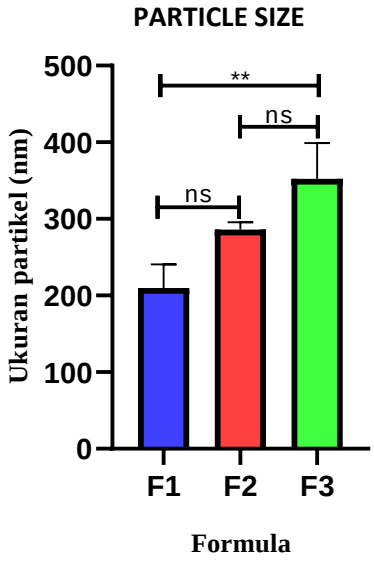

Figure 1. Results of particle size analysis between formulas

The normality test results obtained a p-value > 0,05 so the analysis continued using One-way ANOVA. The results of analysis using Oneway Anova show that the particle size between formulas 1 and 2 is not significantly different with a p-value of 0,0659 (p-value > 0,05). The particle sizes of F1 and F3 show a very significant difference with a p-value of 0,0043 (p-value < 0,05). The difference in particle sizes of F2 and F3 did not show a significant difference with a p-value of 0,1056 (p-value > 0,05). Based on the particle size test results, F1 produces the smallest particle size. This shows that variations in the CMChi concentration used affect particle size.

3.2. Particle Size Distribution

The PI (Polydispersity Index) value is used to measure homogeneity and particle size distribution. Particle size distribution is categorized into monodisperse and polydisperse. PI values range from 0-1. A PI value that is smaller or closer to 0 indicates that the resulting particle size is more homogeneous, if the PI value is close to 1, it indicates that the particle size distribution is increasingly diverse [19],[20].

Table 3. Results of PI testing of nanoparticles from ethanol extract of *B.gymnorrhiza* leaves

Replication	PI ± SD (n=3)		
	F1	F2	F3
1.	0,253	0,306	0,515
2.	0,199	0,389	0,761
3.	0,251	0,397	0,827
Average	0,234	0,364	0,701
SD	0,031	0,050	0,164

The best PI value of nanoparticles was shown in F1 with a PI value of 0,234 ± 0,031. A nanoparticle polydispersity index < 0,2 indicates a monodispersive particle size, namely uniform (homogeneous) particles with a narrow particle size distribution. The PI value in F2 is moderate polydisperse (PI 0,2-0,4) and the PI in F3 is in the polydisperse category PI > 0,4 [14].

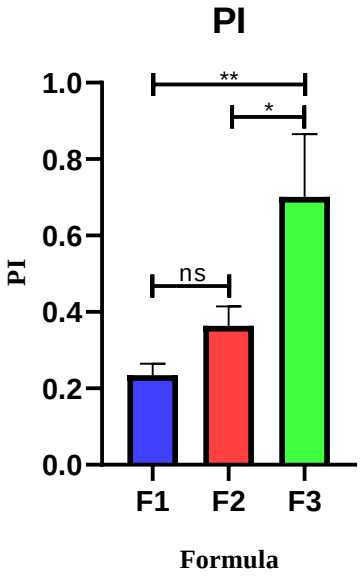

Figure 2. Results of PI analysis between formulas

The resulting PI normality test results obtained a p-value of more than 0,05 so the analysis was continued using One-way ANOVA. The results of the analysis using Oneway Anova, the PI value between F1 and F2 did not show a significant difference with a p-value of 0,3256 (p-value > 0,05). There is a very significant difference in the PI values for F1 and F3 with a p-value of 0,0031 (p-value < 0,05). The PI value between F2 and F3 has a significant difference with a p-value of 0,0151 (p-value < 0,05). The PI value indicates particle instability caused by the agglomeration process. The agglomeration process is influenced by electrostatic energy in the particles and also the influence of Van der Waals forces. The attractive force between electrons causes the distance between one particle and another to get closer and they combine to form larger particles [21].

3.3. Zeta Potential

Zeta potential characterization of *B.gymnorrhiza* leaf extract nanoparticles was carried out on F1 using a Particle Size Analyzer. The zeta potential test results can be seen in the following Table 4.

Table 4. Zeta Potential Measurement Results

Replication	Zeta Potensial (mV) ± SD (n=3)
1	-16,9
2	-16,8
3	-18,6
Average ± SD	-17,43 ± 1,01

The zeta potential value shows the overall charge of a particle in a specific medium. The stability of the nano-dispersed system can be predicted using the zeta potential value. The electrostatic interactions of nanoparticles can be controlled by variations in their surface charge, which can be determined by measuring the zeta potential of the particles [22],[23]. A high zeta value (negative or positive) can prevent particle aggregation by the repulsive force of the dispersed particles so that they can stabilize themselves. On the other hand, a low zeta potential value makes it easier for flocculation (aggregation or sedimentation) to occur between particles

(Das, S., 2011; Suhesti et al, 2017). Zeta potential values ≥ ± 30 mV and 60 mV are considered to be particles with good and excellent stability, respectively. A zeta potential value of ± 30 mV indicates a monodisperse formulation without aggregation, while ± 20 mV tends to have short-term stability, and < 5 mV tends to aggregate quickly [9],[24].

The zeta potential characterization results show quite good values, namely the average zeta potential value is -17,43 ± 1,01 mV. The zeta potential value obtained is negatively charged due to the negatively charged carboxymethyl group (COO^-) which comes from carboxymethyl chitosan. These groups are distributed on the surface of the particles because of their hydrophilic nature [17].

3.4. Particle Morphology

Particle morphology characterization was carried out using a Scanning Electron Microscopy (SEM) tool. The results of morphological observations can be seen in the following image:

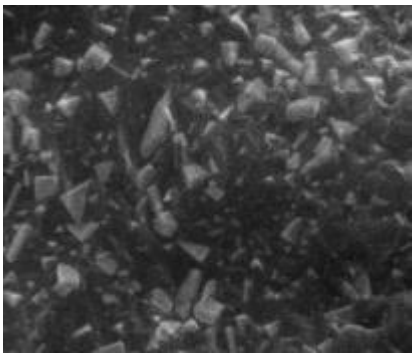

Figure 3. Morphology of *B. gymnorhiza* leaf extract nanoparticles

The nanoparticles were dried using the freeze-drying method and morphology was observed using a Scanning Electron Microscopy (SEM) tool. Based on the results of morphological observations at 10.000 X magnification, a morphological picture of particles with an irregular square shape was obtained. Nanoparticles with a square shape can increase drug solubility, increasing the contact time of the drug with the intestinal cell membrane [25].

3.5. FTIR Analysis

Spreadability testing aimed at Fourier Transform Infra-Red (FTIR) Characterization was carried out to investigate the active substances that have been formed in the nanoparticles. This characterization was carried out by comparing the IR spectrum results between *B.gymnorrhiza* leaf ethanol extract nanoparticles with the IR spectrum of *B.gymnorrhiza* leaf extract nanoparticles. The results of the FTIR analysis can be seen in the following image:

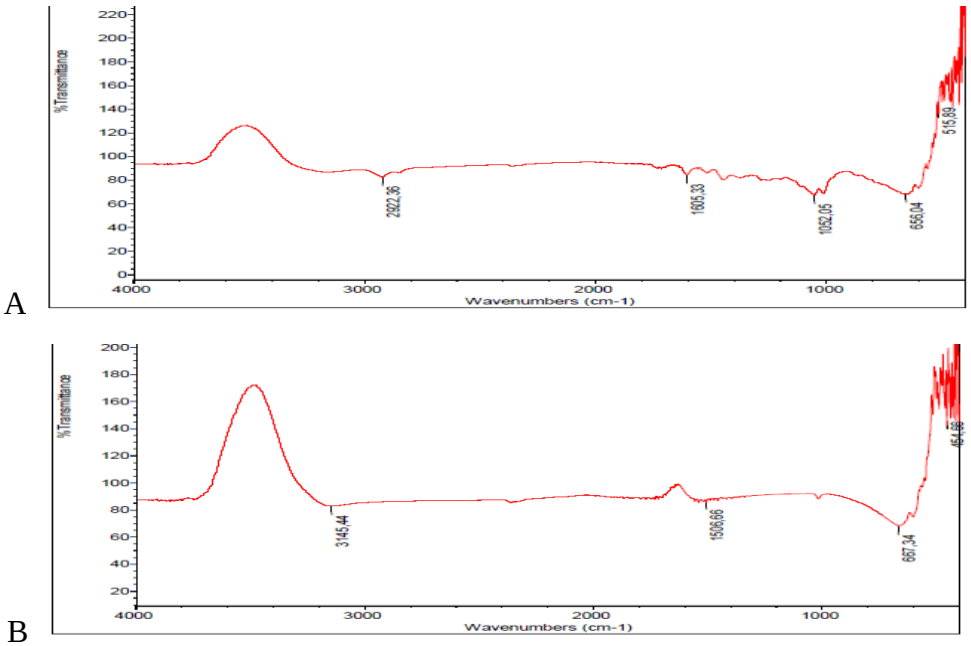

Figure 4. IR spectra of *B. gymnorhiza* leaf extract and *B. gymnorhiza* nanoparticles

The FTIR test results of the ethanol extract of *B.gymnorrhiza* were compared with the IR spectra of *B.gymnorrhiza* nanoparticles, producing a peak of the -OH functional group which was observed at a wave number of 3303 cm^{-1} and had shifted from a wave number of 3336 cm^{-1}. The absorption wavelength of the $-CO^{-}$ (C=O) functional group shifted from 1605,33 cm^{-1} to 1506,66 cm^{-1} The absorption length of the -CH number shifted from 656,04 cm^{-1} to 667,734cm^{-1}.

Table 5. Comparison of Spectra of *Gymnorhiza B* Nanoparticles and Extracts

Ikatan dan daerah serapan gelombang	**Bilangan gelombang (cm^{-1})**	
	Nanopartikel *B. gymnorrhiza*	**Ekstrak *B. gymnorrhiza***
O-H (3200-3600)	3336	3303
C=O (1600-1760)	1644,9	1605,33
C-H alkena (175-995)	667,734	656,04

The similarity between the IR spectrum patterns with several shifts in peak positions indicates the presence of residual carboxymethyl chitosan and $CaCl^2$ in the sample as a capping agent for *B. gymnorhiza* ethanol extract nanoparticles. Therefore, it can be concluded that the polymer is responsible as the capping agent for the synthesized nanoparticles [26],[27]. Based on the FTIR spectrum results, the *B. gymnorhiza* ethanol extract nanoparticle compound has similar functional groups to the *B. gymnorhiza* ethanol extract. This shows that the *B. gymnorhiza* ethanol extract has been loaded in the nanoparticles without providing chemical interactions to form new compounds.

4. Conclusion

4.1. Nanoparticle formulation of EtOH extract from *B. gymnorhiza* F1 leaves using 200 mg carboxymethyl chitosan produces particles with the best physical properties.

4.2. F1 nanoparticles produce small and homogeneous particles, square in shape with a size of 196,5 ± 29,56 nm; The PI is 0,252 ± 0,053 and the zeta potential is -17,43 ± 1,01 mV. The results of FT-IR analysis show that there are similar functional groups between the nanoparticles and *B gymnorhiza* leaf extract.

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