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Original Research Article

Optimization and Characterization of Dayak Onion (*Eleutherine palmifolia* (L.) Merr) Extract Nanoparticles Using Cross-link Method

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ABSTRACT

Dayak onions contain 1,4-Naphtoquinone, which has been shown to possess anticancer activity. Dayak onion extract nanoparticle was formulated to improve its bioavailability using ionic gelation. This research aims to formulate and characterise Dayak onion extract nanoparticles (NPs). The organoleptic properties, pH, entrapment efficiency, particle size, SEM and XRD morphology, and chemical properties of the NPs were investigated by FTIR and DSC. The results showed no aggregation or phase separation; the pH of the formulated NPs was 3.56 ± 0.00. The resulting particle size was 329 ± 87.0 nm, while the entrapment efficiency was 94.84 ± 0.00%. Based on SEM and XRD evaluation, the results obtained were heterogeneous with a non-uniform surface, and the particle structure was amorphous with a degree of crystallinity of 37.98%. Meanwhile, the results of the chemical properties tests using FTIR and DSC agreed with previous studies. There was a shift of functional groups in the IR spectra of the nanoparticles and a shift of melting point in the DSC thermogram, which indicated that Dayak onion extract nanoparticles were successfully formed. Based on data obtained in this study, we conclude that Dayak onion extract can be formulated into nanoparticles with efficient characteristics.

Keywords: Nanoparticles, Cross-link, Chitosan, Tripolifosfat (TPP), Dayak onion

Introduction

Dayak onions (*Eleutherine palmifolia* (L.) Merr) have been experimentally proven to contain antioxidant compounds like phenolics, tannins, and flavonoids. Based on previous research, 96% ethanol extract of Dayak onion bulbs is said to possess high antioxidant and anticancer activity with an IC_{50} value of 22.63 µg/mL and total phenolic content of 6.37% w/w. Meanwhile, other studies with samples taken from different areas showed similar results.[1] Many people have used Dayak onions as a medicinal plant, herbal material, instant powder, or sweets.[2] However, these uses still need improvement, especially regarding the effectiveness of Dayak onions as a medicinal plant, to optimise its therapeutic potential. Nanoparticles are specialised dosage formulations designed to create products in the 10-1000 nm particle size range.[3] The development of Nanoparticles aims to improve the poor solubility and bioavailability of active substances, to protect active substances from environmental degradation to improve their stability, to modify the drug delivery system to enable the drugs to reach specific therapeutic targets, to increase the absorption capacity of macromolecular compounds, and to reduce the potential digestive tract irritation by active substance.[4] Based on these, nanoparticles are considered a solution in using Dayak onions as therapy, considering the characteristics of the active substance in Dayak onions does not dissolve easily in water.

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The main obstacles in the development of nanoparticles are their physical and chemical instability. For example, instability often occurs when making nanoparticle suspensions, such as aggregation, particle fusion, and polymer hydrolysis.[5] To improve the physical and chemical stability of nanoparticles, it is necessary to carry out a freeze-drying procedure to change the nanoparticles from suspension or solution into solid form.

This study aims to formulate Dayak onion extract nanoparticles and to determine the effect of comparing chitosan and TPP concentrations on organoleptic properties, pH, entrapment efficiency, and particle size of formulated nanoparticles. Another variable, such as stirring speed, was also observed for its impact on the organoleptic properties, pH, entrapment efficiency, and particle size of the Dayak onion extract nanoparticles. This evaluation produces an optimum formula that will be evaluated for its functional group characteristics, DSC thermogram, morphology, and degree of crystallinity.

Materials and Methods

Materials

The main ingredient used in this study was the Dayak onion extract obtained from east Kalimantan. The plant sample was identified in the Pharmaceutical Biology Laboratory, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, using macroscopic and microscopic approaches to ensure the authenticity of the sample. A specimen number 074/212/102.20-A/2022 was assigned.

Other formulation ingredients comprised 96% Ethanol (Merck), Chitosan (Himedia), Tripolifosfat (TPP), and acetic acid (Merck).

Dayak Onion Extraction

Extraction was done by dissolving the Dayak onion bulb powdered sample with 96% ethanol in a closed container. The powdered mixture was extracted by sonication for 30 mins using an ultrasonicator (Sonica, Milan, Italy) for 30 minutes. After this, the material was filtered and re-extracted using the same procedure. The combined filtrates were

concentrated to dryness using a rotary evaporator (Heidolph, Germany) to obtain a thick crude extract.[6]

Preparation Dayak onion extract nanoparticles

The Dayak onion extract nanoparticles were prepared using a combined cross-link or ionic gelation with a freeze-drying method. Chitosan solutions were prepared in three concentrations of 0.1%, 0.3%, and 0.5%, and TPP solution with a concentration of 0.1% (Table 1). These three solutions dissolved chitosan in acetic acid using a magnetic stirrer (Heidolph MR-Hei Standard) at 1000 rpm for 30 minutes. Also, TPP (0.1%) was prepared by dissolving TPP powder in distilled water and homogenised using a magnetic stirrer at 1000 rpm for 30 minutes. A total of 10 mg of thick Dayak onion extract was taken and mixed with the chitosan solution previously made. Then, the 0.1% TPP solution was dropped slowly onto the chitosan solution in nine beakers and labelled F1, F2, F3, F4, F5, F6, F7, F8, and F9. At this step, manufacturing optimisation was carried out by varying the speed of the magnetic stirrer. The mixing time was 3 hours. All mixtures were left for 24 hours before freeze-drying was carried out to obtain dry nanoparticles.[1]

Evaluation of Physical and Chemical Characteristics of Dayak Onion Nanoparticles

Organoleptic Characteristics

Organoleptic tests of the formulated NPs included the product's colour and odour. The nine nanoparticle formulas were directly observed for their physical properties. The nanoparticle system should have a transparent colour and a non-pungent odour.[7]

pH Measurement

pH measurement of the NPs was done by dipping the pH meter electrode (pH-2011) into each formula for 30 seconds, and the results were recorded.[7]

Particle Size

Particle size testing was done by inserting Dayak onion extract nanoparticle samples alternately into the PSA instrument (Microtec) cuvette and taking readings.[1]

Entrapment Efficiency

This test was carried out by centrifuging the formed Dayak onion extract nanoparticles at 1500 rpm for 80 minutes. The amount of free Dayak onion extract in the supernatant was measured from the absorbance using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 271 nm with 1,4-naphthoquinone as a comparison. Then, the levels were calculated using a standard calibration curve (linear regression equation).[7]

Evaluation of Functional Group Characteristics

Evaluation of functional group characteristics of the Dayak onion nanoparticles was carried out using the FTIR instrument (Agilent, USA). A total of ± 2 mg of sample was placed in the sample holder on the Fourier Transform Infrared (FTIR) instrument operated at a wave number of 4000-400 cm^{-1}. The resulting spectra were obtained from the MicroLab software on the computer connected to the FTIR instrument.[7]

Evaluation of DSC Thermogram Characteristics

Thermogram characteristics of the formulated Dayak onion extract nanoparticles were evaluated with the Differential Scanning Calorimetry (DSC) instrument. About ±5 mg of each sample was placed in an aluminium pan on a Differential Scanning Calorimetry (DSC) instrument (Linseis, Germany), then heated at a temperature range of 30°-350°C with a temperature increase rate of 10°C per minute.[8]

Evaluation of Morphological Characteristic

The morphological characteristics of the NPs were evaluated with a Scanning Electron Microscopy instrument (Hitachi TM300, Japan). A spatula full of the sample was spread on the glass tube that had been coated and placed in the Scanning Electron Microscope chamber. Then, the particle shape and surface were determined using the SEM instrument.[1]

Evaluation of Particle Structure Characteristics and Degree of Crystallinity

Structural characteristics and degree of crystallinity of the dayak onion Nanoparticles were evaluated with the X-ray diffraction instrument (PAN Analytical X Pert3 Powder). Approximately ± 5 mg of the sample was placed on the sample holder, flattened, and inserted into the sample stage in the X-ray diffraction instrument. Then, the examination was carried out with measurement conditions, namely the target metal Cu Kα filter, a voltage of 40 kV, and a current of 30 mA x-ray source in the 2θ range of 10°-90° C. Analysed through a computer and the diffractogram results were processed using Origin software.[9]

Statistical Analysis

All analyses were performed in triplicate. The data was statistically analysed using the two-way analysis of variance (ANOVA) technique. The level of significance was set as $p < 0.05$. The conditions for this technique to be carried out are that the data must be normally distributed, as proven by Shapiro-Wilk analysis, and homogeneous, as demonstrated by Leven's Test analysis. If one of the conditions is not met, then the analysis uses the Friedman Test Technique.

Results and Discussion

The organoleptic characteristics of Dayak onion extract nanoparticles in this study were observed twice visually. The colloidal system in the form of a nanoparticle dispersion formed as the initial product is shown in Figure 1. Visually, it can be seen that the colour of each formula is influenced by the concentration of chitosan used. The greater the concentration of chitosan used in the formula, the more intense the colour of the resulting dispersion. As with colour, the texture characteristics of each formula also depend significantly on the concentration of chitosan used. Sequentially, the formula's texture becomes thicker from the least concentrated to the highest concentration. However, the nine formulas are similar when viewed in terms of clarity and smell.

Figure 1: Physical Characteristics of F1, F2, F3, F4, F5, F6, F7, F8, and F9

The stirring speed in this study did not have enough influence on the nanoparticles' colour, odour, and texture characteristics. The formula comparing chitosan and TPP produces the same physical characteristics at different stirring speeds. The complete organoleptic test results for the nine formulas are presented in Table 2. As a delivery system, nanoparticles are not targeted to have a certain pH. Generally, the pH will be adjusted when the nanoparticles are transformed into a medicinal dosage form. However, in previous research regarding Dayak onion extract nanoparticles' formulation, the resulting pH range was 3.5–5.5.[1] In this research, the resulting pH value for each formula is shown in Table 3. The table shows that the increase in pH is in line with the increase in chitosan concentration and the ratio of chitosan and TPP used in each formula. Chitosan has a primary amino group with a pKa value of 6.2-7.0, so the higher the concentration of chitosan used in the formula, the higher the pH produced.[8] When viewed from the stirring speed, formulas with the same concentration ratio of chitosan and TPP at different stirring speeds give different results. This shows that the

stirring speed also influences the pH of the resulting product. If we look at the range of previous research findings, F1, F4, and F7 meet the optimum criteria.

Meanwhile, the other six formulas meet these criteria. In other words, the concentration ratio of chitosan and TPP, which can produce a formula with a suitable pH, is 3:1 and 5:1. Two-way ANOVA parametric statistical analysis was carried out for the pH test data in this study. The *p*-value obtained from this test was 0.025 (<0.05), which means that the difference in chitosan concentration and stirring speed significantly affected the pH of the nanoparticles produced.

Similarly, a formula can be said to be nano-sized if the resulting particle size is in the range of 10-1000 nm. In similar research conducted, the size of nanoparticles was 250-450 nm.[1] Another study also stated that, in general, the synthesis of nanoparticles using ionic gelation with chitosan polymer and a cross-linking agent in the form of TPP produces nanoparticles with a size of 200-400 nm.[10] In this research, the particle size and formula polydispersity index are presented in Table 4. If you look at Table 4 and relate it to the results of similar research, F1, F4, F7, and F9 are not included in the optimum criteria. Meanwhile, the other five formulas meet the optimum criteria because they fall into the 250-400 nm range. However, all formulas showed a good polydispersity index of ≤ 0.5 regarding polydispersity. This value indicates that the resulting formula has reasonably good size homogeneity.[11] Several factors influence the particle size of nano chitosan. Previous research reported that the chitosan concentration significantly influences the size of nano chitosan particles. As the chitosan concentration increases, the particle size will also increase. Apart from the chitosan concentration, increasing the TPP concentration in a formula also increases the size of the particles produced. The stirring speed varied in this research, which also impacts the particle size of a nanoparticle system. This follows research that shows that three stirring speeds (500 rpm, 1000 rpm, and 1500 rpm) produce different particle sizes.[11] These differences in results lead to the conclusion that as the stirring speed increases, the particle size will decrease because of the three variations in stirring speed. The homogenised formula shows the smallest particle size at a constant speed of 500 rpm. The Friedman test on the distribution of particle size data gave a p-value of 0.019 (< 0.05), which means that the ratio of chitosan concentration: TPP and stirring speed had a significant effect on particle size (Table 4).

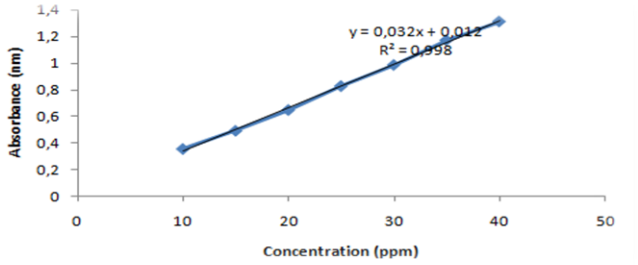

Figure 2: 1,4-Naphthoquinone Standard Curve

Furthermore, the entrapment efficiency of the formulated NPs was obtained from the regression equation (y = 0.032x + 0.012 with an R-value of 0.998) (Figure 2) of standard 1,4-Naphthoquinone solutions. The results of the %EE measurement are presented in Table 5. The nine formulas have relatively good %EE values at > 50%. This shows that most active ingredients used in the nanoparticle formulation have been well encapsulated in the system. The ratio of chitosan: TPP, which gives the best average %EE, was 3:1. This is in line with research conducted in which the mass ratio of chitosan: TPP of 3:1 gave the best adsorption efficiency results. This is because the phosphate groups produced by TPP are balanced with the number of positive amine groups produced by chitosan, so cross-linking occurs well. Meanwhile, in a ratio of 1:1 and 5:1, the amount of positive charge produced by chitosan and the negative charge from TPP was incompatible, reducing the occurrence of cross-links in the nanoparticle system.[12] And concerning the stirring speed, the %EE values produced in this study varied widely. Formulas with the same concentration ratio of chitosan: TPP at different stirring speeds produced different adsorption efficiency values. However, with close observation of the test results data distribution, the stirring speed does not significantly influence it. The difference in values was still clearly visible. This is supported by theory from previous research, which states that stirring speed is directly related to particle size and entrapment efficiency. As the stirring speed increases in nanoparticle synthesis, there will be a decrease in particle size and the adsorption efficiency of the system.[20] The Friedman test on the distribution of entrapment efficiency data gave a p-value of 0.002 (< 0.05), meaning that the ratio of chitosan concentration: TPP and stirring speed significantly affected entrapment efficiency. The optimum formula for Dayak onion extract nanoparticles obtained was F8, which was made with a concentration ratio of chitosan: TPP of 3:1 and a stirring speed of 1250 rpm with evident yellow organoleptic characteristics, a pH of 3.56, a particle size of 329 nm, and an entrapment efficiency of 94.187%. Examining the overall data, the chitosan: TPP concentration ratio 3:1 produces a formula with the best average characteristics. In contrast, for the method, the formula made at a stirring speed of 1000 gives the best average characteristics. The characteristics of functional groups were evaluated in the infrared spectra of the NPs. The infrared spectra of Dayak onion extract, chitosan, TPP, and Dayak onion extract nanoparticles can be seen in Figure 3. The functional groups in the Dayak onion extract used are in Table 6. It can be seen that the Dayak onion extract used in this research has the same content as the literature. In this research, Dayak onion extract nanoparticles were made using chitosan as a polymer and tripolyphosphate (TPP) as a cross-linking agent. The success of the cross-link between chitosan and TPP can be seen in the interaction of functional groups recorded in the infrared spectra. A comparison of the infrared spectra of chitosan, TPP, and Dayak onion extract nanoparticles is shown in Table 7. In the infrared spectrum of Dayak onion extract nanoparticles, there is a peak at a wave number of 3252 $cm^{-1,}$ which indicates an increase in hydrogen bonds. In contrast, the research was located at a wave number of 3360 cm^{-1} with a broad peak. The peak wave number of 1559 $cm^{-1,}$ which indicates the N-H group in chitosan is shifted to a wave number of 1638 cm^{-1}, whereas in the research conducted, the N—H group was located at a wave number of 1580 cm^{-1}, which then shifted to a wave number of 1630 cm^{-1}. Then, a new peak appeared at the wave number of 1541 cm^{-1}, which was the N-O-P stretching group. The new peak was located at a wave number of 1530 cm^{-1}. The presence of the N-O-P stretching group shows the interaction between the amine group in chitosan and the TPP anion to form chitosan Nanoparticles. This indicates that the cross-link between chitosan and TPP in Dayak onion extract nanoparticles was successfully formed.[13]

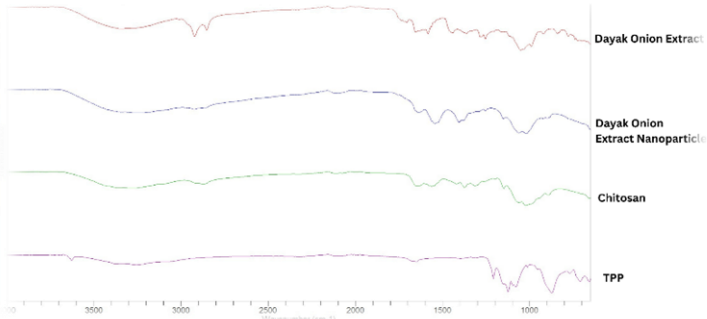

Figure 3: Infrared Spectrum of Chitosan (green), TPP (purple), Dayak Onion Extract (*Eleutherine palmifolia* L. Merr) (red), and Dayak Onion Extract Nanoparticles (blue)

In this study, Dayak onion extract nanoparticles were made to increase the solubility of Dayak onion extract. The loss of the C=O functional group indicates the success of Dayak onion extract carried by a carrier system. In Table 8, the infrared spectra of Dayak onion extract nanoparticles lose the C=O functional group at the wave number 1709 cm^{-1}, which previously appeared in the infrared spectrum of Dayak onion extract. This indicates that Dayak onion extract was successfully carried by the Nanoparticles carrier system, following research conducted, which stated that the loss of the C=O functional group

indicated the success of Dayak onion extract carried by a microsphere carrier system.[13]

The study also evaluated the DSC Thermogram characteristics of the optimum Formula of the NPs. Thermal analysis with DSC was done to analyse the incorporation of Dayak onion extract into the nanoparticle carrier system by looking at the enthalpy changes in the DSC thermogram. Nanoparticles tend to have a lower melting temperature than non-nano-sized materials. The success of forming nanoparticles can be seen from the change in the melting temperature of the nanoparticles compared to their constituent components. The results of the thermal characteristics of Dayak onion extract and Dayak onion extract nanoparticles using DSC are presented in Figure 4. The thermogram of Dayak onion extract shows a glass transition at a temperature of around 63°C. The glass transition temperature (T_g) is one of the essential things in DSC analysis. A material experiences a drastic change in physical shape at the glass transition temperature (T_g), where the shape of the material changes from stiff to soft.[15] Other information obtained from the DSC thermogram of Dayak onion extract is the presence of an endothermic peak at a temperature of 134.4°C, which indicates the melting point of Dayak onion extract.

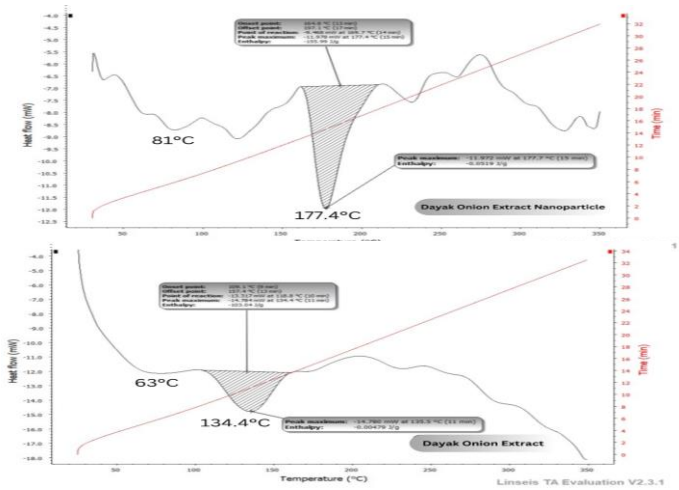

Figure 4: DSC Thermogram of Dayak Onion Extract and DSC Thermogram of Dayak Onion Extract Nanoparticles

In the DSC thermogram of Dayak onion extract nanoparticles above, it is evident that Dayak onion extract nanoparticles begin to undergo drastic changes in shape at a temperature of around 81°C, considered as Dayak onion extract nanoparticles' glass transition temperature (Tg). It can also be seen that Dayak onion extract nanoparticles have an endothermic peak at a temperature of 177.4°C, which shows the melting point of Dayak onion extract nanoparticles. The relationship between the DSC thermogram of Dayak onion extract and Dayak onion extract nanoparticles is presented in Table 9.

This research evaluated thermal characteristics using a DSC instrument on Dayak onion extract and Dayak onion extract nanoparticles. The results of the thermal characteristics are then compared to the thermal characteristics of the nanoparticle components, namely chitosan and TPP, based on the literature. Nanoparticles tend to have a lower melting point than materials that are not nano. According to research, chitosan has an endothermic peak at 304°C and an exothermic peak at 105°C, while tripolyphosphate only has an exothermic peak at 124°C.[14] In this study, Dayak onion extract nanoparticles have a lower endothermic peak than chitosan as its constituent component, namely at a temperature of 177.4°C. In addition, in the DSC thermogram of Dayak onion extract nanoparticles, there is a shift in the melting point compared to Dayak onion extract, chitosan, and TPP. This shift in melting point is thought to be a form of success in Dayak onion extract being absorbed by the nanoparticle carrier system. This was stated in a study on chitosan tetrandine Nanoparticles that the shift in the melting point of the active ingredient in nanoparticles with an active ingredient load indicates the success of the nanoparticles in adsorbing the active ingredient.[8] The endothermic and exothermic peaks of Dayak onion extract, chitosan, and TPP, which are not visible in the Dayak onion extract nanoparticles thermogram, also indicate an interaction between Dayak onion extract and chitosan and TPP. This agrees with research conducted regarding chitosan-TPP nanoparticles.[16] Based on the results of chemical characteristic evaluations (functional groups and DSC thermograms) that have been carried out, Dayak onion extract nanoparticles in this study have adsorbed Dayak onion extract. It could be suggested that the Dayak onion extract nanoparticles possess the anticancer activity contained in the naphthoquinone content in Dayak onion extract.

Again, morphological analysis was performed using a Scanning Electron Microscopy instrument (Hitachi Japan 3000). This evaluation provides information related to drug release properties on nanoparticles where the desired morphological shape is spherical because spherical shapes have greater uptake or absorption than rod shapes.[12] This evaluation is also necessary because the lack of a spherical particle shape can cause contact between particles, which leads to aggregation. The disadvantage during nanoparticle preparation is that it is difficult to control the stability of the dispersion system due to accelerated aggregation and uneven particle size.[17] This study's shape and surface conditions show that dayak onion extract nanoparticles have a heterogeneous shape with a non-uniform surface, as shown in Figure 5. These results agree with previous research using the same materials and methods. The morphological results obtained are in the form of a spherical shape but have a rough surface, which is believed to be associated with the poor preparation process of coating with gold metal. The results of a non-uniform particle shape that is not symmetrical could be due to the drying process that releases liquid from the mixture, making the shape irregular. In addition, differences in the results obtained are also possible due to several factors, such as the materials used, the concentration of chitosan and TPP, the manufacturing method or process, and the pH. Particles that are not uniform and clustered together indicate agglomeration, which may be caused by particle size, H_2O on the surface of nanoparticles, or the presence of other oxide elements. A study explained that the higher the chitosan concentration, the larger the particle size. This is due to the increased interaction of chitosan, causing the aggregates formed to merge into larger particles. In addition, the stirring speed also affects the particle size. Increasing the stirring speed will accelerate the spread of tripolyphosphate, thus causing aggregates.

In this study, particle structure characteristics and degree of crystallinity were evaluated using the X-ray diffraction instrument. The degree of crystallinity is defined as a quantity that states the amount of crystal content in a material by comparing the area of the crystal curve with the total area of amorphous and crystals.[9] The diffractogram results of dayak onion extract nanoparticles obtained were analysed using origin software. The results of the diffractogram of the optimum formula of dayak onion extract nanoparticles in Figure 6 show that there is no sharp peak, but there is a broad peak at the 2θ angle in the range of 22.86°-36.31° with a peak located at 28.273°. The area of this peak was found to be 13732.72, and the total area was 36154.11, which is presented in Table 11. The degree of crystallinity is calculated using the crystallinity degree formula through the origin and the Excel programs, as follows:

$$\text{Crystallinity} = \frac{(Crystalline\ area\ fraction)}{(Crystalline\ area\ fraction + amorphous\ area\ fraction)} \times 100$$

$$\text{Crystallinity} = \frac{13732,72}{36154,11} \times 100 = 37,98\%$$

From these calculations, a crystallinity index value of 37.98% was obtained. The disappearance of sharp peaks or a new change to an amorphous structure indicates that the drug compound has been encapsulated well in the nanoparticle system and is predicted to increase the solubility and bioavailability of the drug compound.[19] However, the diffractogram results of chitosan nanoparticles and dayak onion extract nanoparticles with microspheres dayak onion extract have different results, where the microspheres show a crystal structure. In addition, there is no specific peak typical of chitosan in the nanoparticles of dayak onion extract.

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Table 1: Dayak onion nanoparticles formula (*Eleutherine palmifolia* (L.) Merr.)

Formula Code	Amount of Ingredients in Formulation			Chitosan concentration	Ratio of chitosan : TPP concentration	Stirring Speed
	Dayak Onion Extract	Chitosan Solution	0.1% TPP Solution			
F1	10 mg	20 ml	4 ml	0.1%	1 : 1	750 rpm
F2	10 mg	20 ml	4 ml	0.3%	3 : 1	750 rpm
F3	10 mg	20 ml	4 ml	0.5%	5 : 1	750 rpm
F4	10 mg	20 ml	4 ml	0.1%	1 : 1	1000 rpm
F5	10 mg	20 ml	4 ml	0.3%	3 : 1	1000 rpm
F6	10 mg	20 ml	4 ml	0.5%	5 : 1	1000 rpm
F7	10 mg	20 ml	4 ml	0.1%	1 : 1	1250 rpm
F8	10 mg	20 ml	4 ml	0.3%	3 : 1	1250 rpm
F9	10 mg	20 ml	4 ml	0.5%	5 : 1	1250 rpm

Table 2: Organoleptic Tests Result

Formula	Colour	Texture	Odor
F1	Pale yellow, clear	Aqueous	Sour
F2	Yellow, clear	Thick	Sour
F3	Deep yellow, clear	Thick	Sour
F4	Pale yellow, clear	Aqueous	Sour
F5	Yellow, clear	Thick	Sour
F6	Deep yellow, clear	Thick	Sour
F7	Pale yellow, clear	Aqueous	Sour
F8	Yellow, Clear	Thick	Sour
F9	Deep yellow, Clear	Thick	Sour

Table 3: pH Value of Dayak Onion Extract Nanoparticles

	Formula	Mean ± SD*
	F1	3.3633 ± 0.0057
	F2	3.5200 ± 0.0100
*data	F3	3.5500 ± 0.0000
	F4	3.3700 ± 0.0000
	F5	3.5433 ± 0.0152
	F6	3.5600 ± 0.0000
	F7	3.3733 ± 0.0057
	F8	3.5667 ± 0.0057
	F9	3.5900 ± 0.0200

replicated three times ± SD

Table 4: Particle Size Value of Dayak Onion Extract Nanoparticles

	Formula	Particle size (nm)*	
	F1	198.7 ± 52.3	0.18
	F2	325.5 ± 167.0	0.57
*data	F3	350 ± 117.5	0.22
	F4	111.6 ± 58.8	0.22
	F5	267.1 ± 105.0	0.22
	F6	345.8 ± 128.2	0.25
	F7	4297 ± 915.3	0.50
	F8	329 ± 87.0	0.20
	F9	164 ± 16.1	0.24

replicated three times ± SD

Table 5: Entrapment Efficiency Measurement Result

	Formula	EE (%) ± SD*
	F1	88.93 ± 0.009
	F2	94.40 ± 0.003
*data	F3	90.29 ± 0.009
	F4	89.82 ± 0.006
	F5	94.63 ± 0.003
	F6	91.10 ± 0.003
	F7	89.69 ± 0.013
	F8	94.84 ± 0.000
	F9	81.58 ± 0.325

replicated three times ± SD

Table 6: Functional groups in the infrared spectra of Dayak onion (*Eleutherine palmifolia* (L.) Merr) extract

Functional Group	Wavenumber (cm^{-1})	Reference Wavenumber (cm^{-1})	Reference
O–H	3350 and 3008	3437 and 3430	21
Aromatic C–H	2922 and 2853	2924 and 2852	21
C=O	1709	1728	21
Aromatic C=C	1654 and 1448	1656 and 1458	21
C–O	1053	1013	13

Table 7: Functional Groups of Chitosan, TPP, Chitosan-TPP Nanoparticles, and Dayak Onion Extract Nanoparticles (*Eleutherine palmifolia* (L.) Merr)

Sample	Wavenumber (cm^{-1})	Reference Wavenumber (cm^{-1})	Notes
Chitosan	3358	3360	N–H stretching vibration and –OH stretching vibration
	2873	2870	C–H stretching vibration
	1654	1650	C=O of amide group –CONH
	1559	1580	Bending vibration of N–H
	1420	1430	Bending vibration of O–H deformation
	1375	1370	Bending vibration of C–H
	1060 and 1023	1080 and 1030	Stretching vibration of C–O
TPP	1209	1210	Stretching vibration of P=O
	1123	1130	Stretching vibration of O–P=O
	1097	1090	Stretching vibration of PO_3
	874	888	Stretching vibration of P–O–P bridge
Chitosan-TPP Nanoparticles	3252	3360 *broad*	Bending vibration of N–H and stretching vibration of –OH
	1541	1530	Stretching vibration of N–O–P
	1638	1630	Bending vibration of N–H

Table 8: Comparison of Functional Groups in the Infrared Spectrum of Dayak Onion Extract and Dayak Onion Extract Nanoparticles (*Eleutherine palmifolia* L. Merr)

Sample	Functional Group	Wavenumber (cm^{-1})
Dayak Onion Extract	O–H	3350 and 3008
	Aromatic C–H	2922 and 2853
	C=O	1709
	C–O	1654 and 1448
Dayak Onion Extract	O–H	3252 cm^{-1}
	C–H	2924 cm^{-1}
	C=C	1638 cm^{-1}
	Bending N–H	1559 cm^{-1}
	C–N	1149 cm^{-1}
	C–O	1062 cm^{-1} and 1019 cm^{-1}

Table 9: Thermal Characteristics of Chitosan, TPP, Dayak Onion Extract, and Dayak Onion Extract Nanoparticles

Sample	Endothermic		Exothermic	
	T_P (°C)	ΔH (J/g)	T_P (°C)	ΔH (J/g)
Chitosan*	304	435.1	105	-807
TPP	-	-	124	-221.7
Dayak Onion Extract	134.4	-103.04	-	-
Dayak Onion Extract Nanoparticles	177.4	-155.99	-	-

Table 10: XRD Data Results of Dayak Onion Extract Nanoparticles

Index	Area	AreaIntgP (%)	Row index	Beginning X	Ending X	FWHM	Center	Height
1	13732.7223	37.984	1094	22.859	36.31	13.436	28.27	1165.94

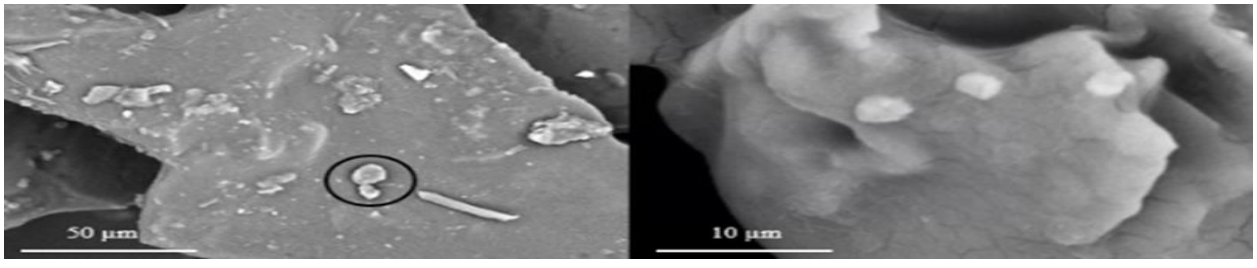

Figure 5: Morphology of Dayak Onion Extract Nanoparticles with 1200x and 7000x Magnification

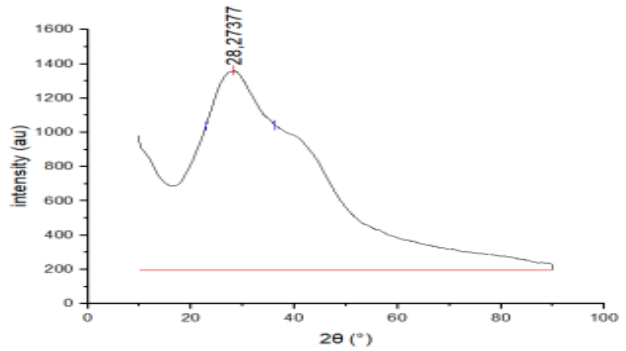

Figure 6: Nanoparticles diffractogram results of Dayak Onion Extract

Conclusion

Factors influencing the physical characteristics of Dayak onion extract nanoparticles are the ratio of chitosan and TPP for each formula and the stirring speed used during nanoparticle synthesis. The optimum Dayak onion extract nanoparticles formula was F8 with a characteristic clear yellow colour, average pH value of 3.56, an average particle size of 329 nm, entrapment efficiency of 94.187%; FTIR results show that there is an absorption peak of the N-O-P functional group at wave number 1541 cm^{-1}, the disappearance of the C=O group, and a shift in the functional group in the FTIR infrared spectrum; a shift in the melting point in the DSC thermogram; the morphology of the resulting shape is heterogeneous with a non-uniform surface; and testing using XRD shows that the particle structure is amorphous and has a degree of crystallinity value of 37.98%.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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