

Influence of Chitosan Concentration on Characteristic of Microspheres Delivery System Prepared from *Eleutherine palmifolia* (L.) Merr. Extract

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ABSTRACT

Background: *Eleutherine palmifolia* (L.) Merr. (*E. palmifolia*) is a medicinal plant containing naphthoquinone, isoliquiritigenin, and oxyresveratrol compound. This study aims to formulate and characterize the *E. palmifolia* microspheres delivery system using chitosan variation as the polymer. **Methods:** The preparation method was the solvent evaporation method. The microspheres delivery system was performed by variation of chitosan concentration in each formulation 0.637% (F1), 1.275% (F2), and 1.912% (F3). The physical and chemical characterizations include Entrapment Efficiency (EE), yield percentage, particle size, particle morphology, FTIR analysis, X-Ray Diffraction (XRD) analysis, and Differential Thermal Analysis (DTA) had been evaluated. **Results:** The results showed that chitosan concentration variation affected the microsphere's physical and chemical characteristics. Variations in the concentration of chitosan polymer (0.637%, 1.275% and 1.912%) had an effect on the physical characteristics of the microspheres of the resulting Dayak onion bulb extract. The higher the concentration of chitosan used, the higher the entrapment efficiency and the yield yield will increase. Meanwhile, in observing the particle size, the higher the concentration of chitosan used, the smaller the particle size produced. The optimal concentration of chitosan as a polymer at concentration variations of 0.637%, 1.275% and 1.912% to provide good physical characteristics of the Dayak onion bulb extract microspheres was shown in formula 3 with a chitosan concentration of 1.912%. **Conclusion:** The better characteristic was obtained upon higher chitosan concentration. The best physical characteristics of *E. palmifolia* extract microspheres were obtained from chitosan concentration at 1.912% (F3).

Key words: *Eleutherine palmifolia*, Chitosan, Characteristic, Microspheres, Delivery system.

INTRODUCTION

Eleutherine palmifolia (L.) Merr. (*E. palmifolia*) is a unique Kalimantan plant, which is used by Dayak people as a medicine.¹ Some studies have proven that *E. palmifolia* contains secondary metabolite compounds of the naphthoquinone category and its derivatives such as *elecacin*, *eleutherol*, and *eleutherinon* that contain bioactivity as anticancer.² The study claims that *E. palmifolia* could be the potential to as one of the anticancer medicines. *E. palmifolia* can be developed into a pharmacy; one of them is a provision of the tablet. However, a provision of the table with a conventional drug delivery system has some weaknesses, including low bioavailability, a short half-life causing repetitive drug administration, and the possibility of decreasing the patient's compliance.³ One of the ways to overcome the problem is by developing a controlled drug delivery system.

The controlled drug delivery system is one of the drug delivery systems that can increase the drug half-life, reduce the side effect, reduce the frequency of the drug's dose administration, and increase the patients' obedience during a therapy session.⁴ Microspheres are solid particles that form. They contain active substances, spreading homogeneously with a range of 1-1000 μm particle size.⁵ Microspheres' drug delivery system has some strengths, such as providing a more

prolonged and more constant therapy effect, increasing the drugs' bioavailability, reduce the frequency of dose administration. as a result, As can increase the patients' obedience, transmit the drugs to a specific body organ leading to reduce the possibility of side effects to another organ and able to provide a controlled drug release.⁶ Microspheres are used as controlled drug delivery systems for various applications, including chemotherapy, cardiovascular disease, hormone therapy, therapeutic protein delivery, and vaccine development.

To synthesize microspheres, the drug delivery system needs a polymer transport system including both natural polymers (e.g., gelatin and chitosan) and synthetic polymers (e.g., polylactide polyacrylanocrylate and others).⁷ The polymer used in this study is a chitosan polymer. Chitosan has a bioadhesive characteristic in the digestive tract (mucoadhesive); hence it can prolong the drug's retention time and release time.⁸ The use of chitosan polymer in microspheres drug delivery systems influences the percentage value of the produced entrapment efficiency.⁹ Chitosan is considered one of the most valuable polymers for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity, and antitumor properties. The use of biodegradable polymers minimizes the possibility of toxicity problems but does produce byproducts that must be tolerated without adverse reactions.

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Based on the explication above, further research concerning *E. Palmifolia* extracts formulation in microspheres drug delivery system using chitosan as a polymer. For this study, the physical and chemical characteristics of the microspheres had been investigated, such as Entrapment Efficiency (EE), yield, particle size analyzer (PSA), Scanning Electron Microscope (SEM), FTIR, X-Ray Diffraction (XRD), and Differential Thermal Analysis (DTA).

MATERIAL AND METHODS

Extract preparation

The *E. palmifolia* was extracted three times with 500 mL ethanol at ambient temperature through sonication Q2400 (Sonica, USA) at 10 min intervals. About 25 grams of the sample was dissolved in 500 mL of 96% ethanol at 1:20. The filtrate was then separated from the solvent using a rotary evaporator (Heidolph, Germany).

Herbal material

The *E. palmifolia* samples were purchased from vendors in East Kalimantan and identified at the Materia Medica in Batu, East Java, Indonesia, with the accession number 074/342A/102.7/2018. The specimens were then stored in the pharmacognosy Laboratory of the Pharmacy Department, Maulana Malik Ibrahim, State Islamic University of Malang.

Materials

Chitosan was purchased from Biotech. Acetone was purchased from Smart Lab. Paraffin liquid purchased from Bratachem. Span 80 was purchased from Bratachem. Petroleum ether was purchased from Bratachem. Ethanol 96% purchased from Bratachem.

Data analysis

Analysis of the data used in the study was carried out on testing the characteristics of the microspheres by measuring 3 times. The data obtained were analyzed using SPSS statistics 20.

Preparation of microspheres delivery system from *E. palmifolia* extract

Formulation

Three types of formulations of microspheres drug delivery systems were fabricated with a concentration comparison between active ingredients and different chitosan polymers to produce a weight of 117.669 grams. Microsphere's drug delivery system was expected to have a particle size of 1-1000 μm , a spherical morphology with a flat surface, and the percentage value of entrapment efficiency close to 100%. The microsphere's delivery system formulation design is presented in table 1.

Table 1: Formulation microspheres of *E. palmifolia* extract.

| Materials | Formulation Concentration % (w/w) | | |
|-----------------------------|-----------------------------------|----------|----------|
| | F1 (1:1) | F2 (1:2) | F3 (1:3) |
| <i>E.palmifolia</i> Extract | 0.637 | 0.637 | 0.637 |
| Chitosan | 0.637 | 1.275 | 1.912 |
| Acetone | 16.113 | 16.113 | 16.113 |
| Paraffin liquid | 64.248 | 64.248 | 64.248 |
| Span 80 | 0.772 | 0.772 | 0.772 |
| Petroleum ether | 17.591 | 16.954 | 16.317 |
| Total Amount | 100 | 100 | 100 |

Note: Formulation 1 (F1); Formulation 2 (F2); Formulation 3 (F3)

Procedure

The preparation method used in this study was the solvent evaporation method. Firstly, *E. palmifolia* extract was dissolved in acetone solvent as the internal phase. Afterward, Chitosan polymer was mixed into the extract solution. The mixture was then emulsified in an external phase containing span 80 and paraffin liquidum. Furthermore, the mixture was magnetically stirred with 1200 rpm spin for two hours and filtered by using Whatman number 41 filter paper to collect the formed microspheres. During the filtration process, the collected microsphere is cleansed using petroleum ether to remove the oil phase in the emulsification stage. Subsequently, the collected microsphere was dried in the oven at a temperature of 70°C for two hours. The as-prepared microspheres showed an average weight of formulation 1=1.097 grams \pm 0.019, formulation 2 = 1.882 grams \pm 0.024, and formulation 3 = 2.706 gram \pm 0.015.

Characterization

Entrapment efficiency: Entrapment efficiency is one of the success factors in microspheres preparation. Per cent entrapment efficiency indicates how much drug is trapped in microspheres particles. entrapment efficiency was determined by weighing up the microsphere, which is equal to 50 mg of *E. palmifolia* extract, demineralized water is added to it until the volume reaches 10 mL. The measurement of entrapment efficiency percentage was performed through spectrophotometer UV 1800 (Shimadzu, Japan) at a wavelength of 340.5 nm. The result of separation in the form of *E. Palmifolia*, trapped in the microspheres delivery system, will be precipitated after it is separated using a centrifuge 32 (Hettich Rotofix, USA) at 2500 rpm for 45 minutes. *E. palmifolia* extract, which is not stuck in the microspheres delivery system, will be dispersed in *equates* as a supernatant. Afterward, the supernatant is diluted up to a concentration of 500 rpm. The next step is a free drug concentration measurement in the water phase in the microspheres delivery system dispersion, and it uses *equates*. It is measured using the formulation $EE (\%) = [(Ct-Cf)/Ct] \times 100\%$. Ct: the number of medicinal ingredients used. Cf: the number of medicinal ingredients in the water phase. Three repeated readings were performed for the sample.¹⁰

Yield percentage: Yield is a microsphere characterization that portrays the efficiency of the preparation method used to produce microspheres. The higher concentration of polymer used in the manufacturing of microspheres, the percent yield produced will be obtained.¹¹ Determining the yield percentage of each formulation was determined by measuring the amount of the produced microspheres compared to the microsphere drug theoretically. Percent yields close to 100% indicate that the method used in the preparation of microspheres results in the maximum and most efficient microspheres. The yield percentage was calculated the obtained microspheres weight (mg)/the theoretical microspheres weight (mg) \times 100 %. Three repeated readings were performed for the sample.

Particle size: The particle size of the emulsion formed after the reconstitution of microspheres was determined by dynamic light scattering Nanowave II (Microtrac, USA). The formulations were diluted at a ratio of 1:10 w/w with water and mixed well for 1 min. The diluted samples were transferred into cuvettes (model nano PTFE). A relative refractive index of 1.20 (ratio of the indices between the oil and water phases) was used. Three repeated readings were performed for the sample.

Particle morphology: The form and morphology of the microsphere were investigated using a Scanning Electron Microscope (SEM) TM300 (Hitachi, Japan). The sample was placed on the glass stub and measured by a scanning electron microscope with a voltage of 20 kV and 0.1 mmHg chamber pressure with 500 and 1000 times magnifications.

X-Ray Diffraction (XRD): Microsphere forming analysis using X-Ray Diffraction (XRD) is executed to find out the formation of microspheres made by observing the peak of the obtained using X-Ray Diffraction (Shimadzu, Japan). For examination, the sample is placed on the sample holder and is spread evenly to prevent any particle orientation during sample preparation.

Different Thermal Analysis (DTA): Microspheres delivery system analysis using Different Thermal Analysis (DTA) FP90 TA Cell (Mettler Toledo, USA) was carried out to know the melting point of the prepared microspheres delivery system. The melting point examination was scanned at 30°C up to 300°C with an acceleration of temperature increase of 20°C per minute.

RESULTS AND DISCUSSION

Microspheres are solid particles and contain active ingredients that are evenly distributed in them with a particle size range of 1-1000 nanometer.⁵ This microsphere delivery system aims to provide a longer therapeutic effect, increase drug bioavailability, reduce the frequency of drug administration so as to improve patient compliance, deliver drugs to specific organs and be able to provide controlled drug release.⁶ This microsphere delivery system consists of an active ingredient and a polymer as a carrier.

The active ingredient used in this research is the extract of Dayak onion bulbs. Dayak onion bulbs are known to have several benefits as a medicine for various types of diseases, one of which is for the treatment of cancer. Several studies have proven that Dayak onion bulbs are known to contain secondary metabolites of the naphthoquinone group and their derivatives such as elecacin, eleutherol and eleutherinone which are known to have anticancer bioactivity. The polymer used in this research is chitosan polymer. Chitosan is a natural polymer produced from deacetylation of chitin, non-toxic, non-irritating, biocompatible and biodegradable. In addition, other components used in the manufacture of this microsphere delivery system consist of acetone used as the internal phase, paraffin liquidum as the external phase, span 80 as a surfactant and petroleum ether as a washing agent.

Characterization of microspheres *E. palmifolia*

Drug entrapment efficiency: Table 2 below is the result of entrapment efficiency with a comparison of active ingredients and chitosan formulation (1:1), formulation 2 (1:2), formulation 3 (1:3). The result of entrapment efficiency percentage indicated that the higher the chitosan concentration could increase the yield of entrapment efficiency percentage, due to greater the chitosan capability to absorb the active ingredients into microspheres.¹² This also shows that the higher the entrapment efficiency produced, the greater the chitosan ability to protect active substances from external influences that can damage the active substances, hence the bioavailability of active substances will also increase.¹³ Statistical analysis shows that there are significant differences in this test in each of the tested formulas. This indicates that there is an effect of chitosan concentration on the entrapment efficiency of microsphere delivery system prepared from *Eleutherine palmifolia*(L.) Merr. extract.

Yield percentage: Table 3 below is the result of yield percentage with a comparison of active ingredients and chitosan formulation (1:1), formulation 2 (1:2), formulation 3 (1:3). The yield percentage showed that the preparation of the microsphere's delivery system using a solvent evaporation method reached a maximum number of microspheres with almost 100%.¹⁴ The partial loss of microspheres (decrease in percent yield) could be caused by an improper filtering process.¹⁵ Statistical analysis shows that there are significant differences in this test in each of the tested formulas. This indicates that there is an effect of chitosan concentration on the yield percentage of microsphere delivery system prepared from *Eleutherine palmifolia* (L.) Merr. extract. It indicates

that the increase in chitosan concentration in microspheres delivery systems could affect the yield percentage of microspheres.

Particle size: Table 4 below is the result of particle size with a comparison of active ingredients and chitosan formulation (1:1), formulation 2 (1:2), formulation 3 (1:3). Particle size observation showed that the microspheres delivery system of Dayak onion tubers extracts of formulation 1, formulation 2 and formulation 3 had particle sizes of 4.045 $\mu\text{m} \pm 1.173$, 5.853 $\mu\text{m} \pm 0.237$ and 1.487 $\mu\text{m} \pm 0.413$. This is following the particle size requirements stipulated that microspheres have a size range of 1-1000 μm . Statistical analysis shows that there are significant differences in this test in each of the tested formulas. This indicates that there is an effect of chitosan concentration on the particle size of microsphere delivery system prepared from *Eleutherine palmifolia* (L.) Merr. extract. This shows that increasing the concentration of chitosan in the microsphere's delivery system can affect the particle size. The higher the concentration of chitosan, the lower the particle size produced.

Particle morphology: The result revealed that formulation 1 in Figures 1A and 1D and formulation 2 in Figures 1B and 1E had a non-spherical form with an irregular surface, while formulation 3 in Figures 1C and 1F had spherical form and flat surface. The non-spherical form with the irregular surface in formulations 1 and 2 might be caused by the low viscosity of chitosan. It causes the formation of microspheres structure to be not strong enough to shrink.¹⁶

Fourier Transfer Infrared (FTIR) analysis: FTIR analysis was carried out to prove that the *E. Palmifolia* extract was successfully trapped into chitosan-based microspheres. This is indicated by the loss of the absorption band in the wavenumber area of 1647 cm^{-1} , which is the -C=O functional group in the *E. palmifolia* extract. Whereas in the FTIR spectrum of chitosan, formulation 1, formulation 2, and formulation 3. The absorption band appears in the area of wave number 1578 cm^{-1} , which is the bending vibration (NH bending) of the primary amine functional group (-NH₂) and absorption area in the 1319 cm^{-1} and 1149 cm^{-1} with low intensity where the C-N stretching vibrations [16]. The absorption band (range vibration) of the primary amine functional group in the area 3500-3300 cm^{-1} both in chitosan, F1, F2, and F3 are not visible because it overlaps with the absorption of the -OH functional group in the 3352 cm^{-1} region (chitosan and F1)

Table 2: The result of entrapment efficiency.

| Formulation | Average of EE (%) \pm SD* |
|-------------|-----------------------------|
| F1 (0.637%) | 95.707 \pm 0.066 |
| F2 (1.275%) | 96.967 \pm 0.051 |
| F3 (1.912%) | 98.031 \pm 0.074 |

Note : *SD value of 3 times replication

Table 3: The result of the yield percentage.

| Formulation | Weight of Microspheres Obtained | Weight of Microspheres Theoretical | Average of Yield (%) \pm SD* |
|-------------|---------------------------------|------------------------------------|--------------------------------|
| 1 (0.637%) | 1.097 gram | 1.500 gram | 73.155 \pm 1.234 |
| 2 (1.275%) | 1.882 gram | 2.250 gram | 83.659 \pm 1.072 |
| 3 (1.912%) | 1.706 am | 3.000 gram | 90.200 \pm 0.483 |

Note : *3 times replication of SD value

Table 4: The result of particle size.

| Formulation | Average of Particle Size \pm SD* |
|-------------|------------------------------------|
| 1 (0.637%) | 4.045 $\mu\text{m} \pm 1.173$ |
| 2 (1.275%) | 5.853 $\mu\text{m} \pm 0.237$ |
| 3 (1.912%) | 1.487 ± 0.413 |

(*) the value of SD 3 times replication

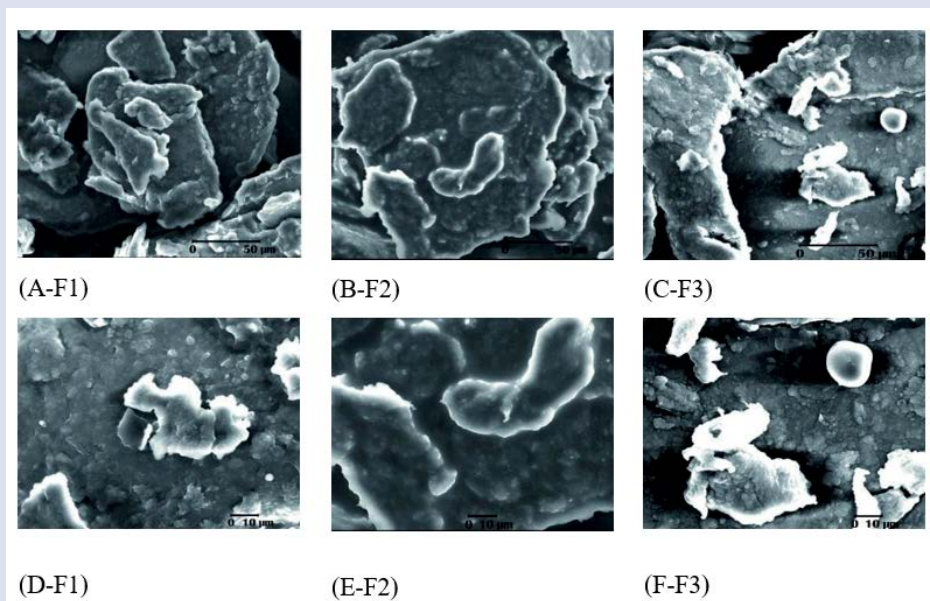


Figure 1: Particle morphology (A) Formulation 1 (B) Formulation 2 (C) Formulation 3 at a magnification of 500 times (D) Formulation 1 (E) Formulation 2 (F) Formulation 3 at a magnification of 1000 times using Scanning Electron Microscope (SEM).

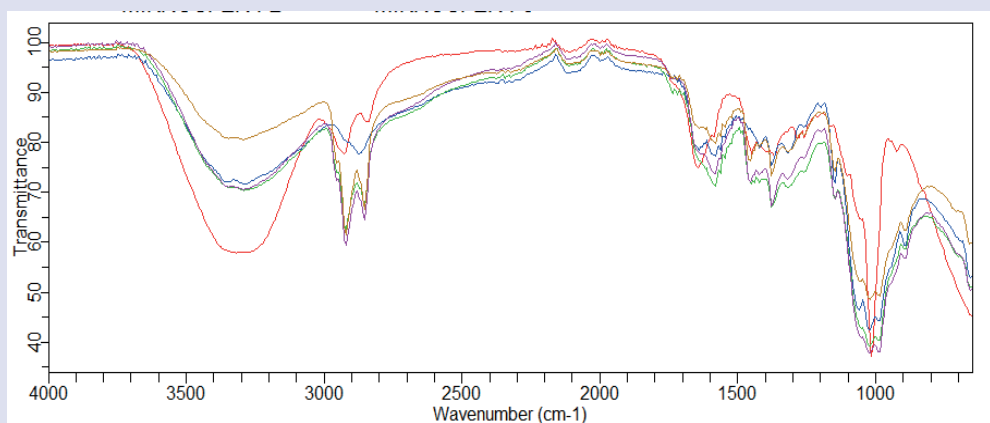


Figure 2: Infrared Spectrum of *E. palmifolia* (red), Chitosan, (blue), Formulation 1 (yellow), Formulation 2 (green), Formulation 3 (purple).

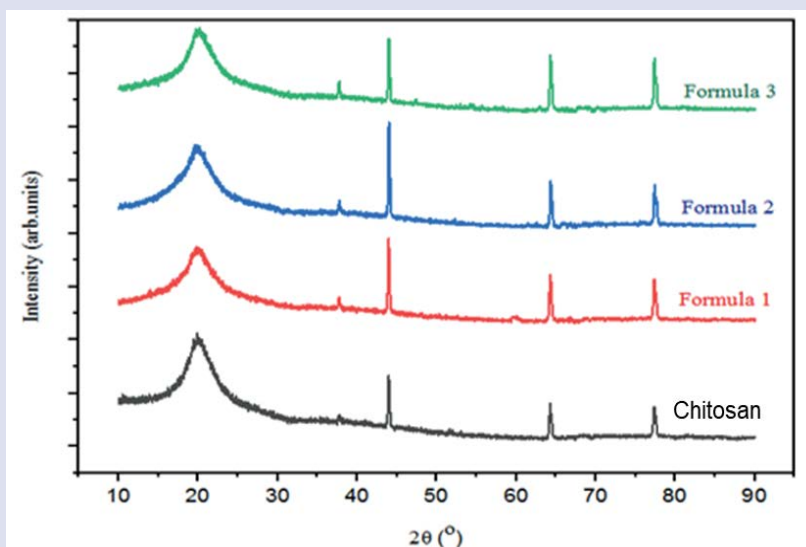


Figure 3: X-Ray Diffraction Spectra of Chitosan (—), Formulatin I (—), Formulation 2 (—), Formulation 3 (—).

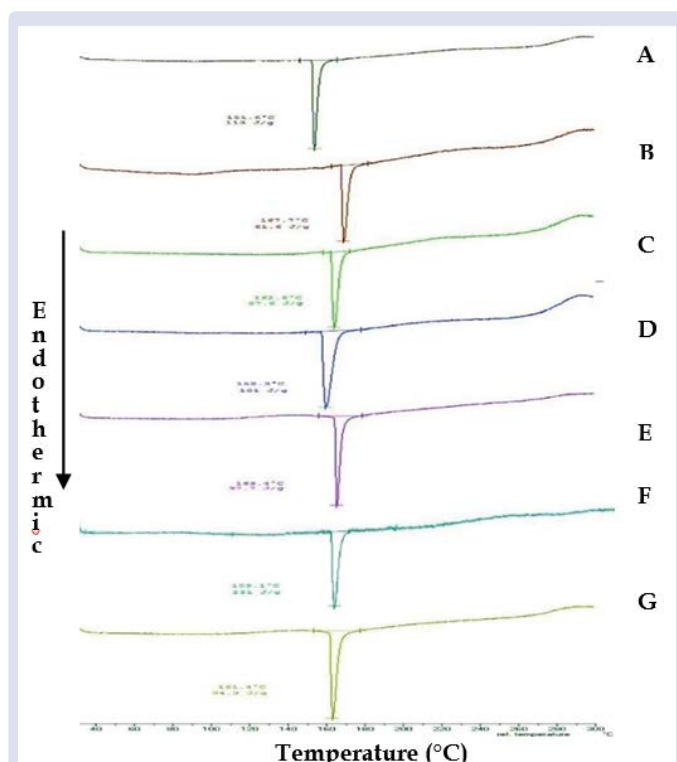


Figure 4: The Differential thermal analysis (DTA) of (A) chitosan, (B) microspheres F1 without *E. palmifolia*, (C) microspheres F2 without *E. palmifolia*, (D) microspheres F3 without *E. palmifolia*, (E) microspheres F1 (F) microspheres F2 (G) microspheres F3.

Table 5: The results of the analysis of the formation of microspheres by FTIR.

| Sample | Wave Numbers | Functional groups |
|----------------------|---|--------------------|
| <i>E. palmifolia</i> | 3285 cm ⁻¹ | O-H |
| | 2927 cm ⁻¹ dan 28... | C-Hsp ³ |
| | 1647 cm ⁻¹ | C=O |
| | 1449 cm ⁻¹ | C=C |
| | 1013 cm ⁻¹ | C-O |
| | 3352 cm ⁻¹ | O-H vs N-H |
| | 2871 cm ⁻¹ | C-H |
| Chitosan | 1578 cm ⁻¹ | N-H |
| | 1375 cm ⁻¹ | -CH ₃ |
| | 1319 cm ⁻¹ dan 1149 cm ⁻¹ | C-N |
| | 1060 cm ⁻¹ dan 1023 cm ⁻¹ | C-O |
| | 3352 cm ⁻¹ | O-H |
| Formulation 1 | 2922 cm ⁻¹ dan 2853 cm ⁻¹ | C-Hsp ³ |
| | 1578 cm ⁻¹ | N-H |
| | 1375 cm ⁻¹ | -CH ₃ |
| | 1149 cm ⁻¹ | C-N |
| | 1060 dan 1023 | C-O |
| Formulation 2 | 3358 cm ⁻¹ | O-H |
| | 2922 cm ⁻¹ dan 2853 cm ⁻¹ | C-Hsp ³ |
| | 1578 cm ⁻¹ | N-H |
| | 1377 cm ⁻¹ | -CH ₃ |
| | 1149 cm ⁻¹ | C-N |
| Formulation 3 | 1060 dan 1023 | C-O |
| | 3358 cm ⁻¹ | O-H |
| | 2922 cm ⁻¹ dan 2853 cm ⁻¹ | C-Hsp ³ |
| | 1578 cm ⁻¹ | N-H |
| | 1377 cm ⁻¹ | -CH ₃ |

Table 6: The Result of XRD analysis.

| Sample | X-Ray Diffraction Peaks at 2θ |
|----------------------|---|
| <i>E. palmifolia</i> | 15.809, 16.776° and 17.750° |
| Chitosan | 19.86°, 37.72°, 43.96, 64.28° and 77.36° |
| Formulation 1 | 19.86°, 37.72°, 43.96°, 64.28° and 77.36° |
| Formulation 2 | 19.86°, 37.76°, 43.98°, 64.30° and 77.42° |
| Formulation 3 | 19.78°, 37.72°, 43.98°, 64.30° and 77.40° |

Table 7: The result of DTA analysis.

| Formulation | Melting Point (°C) | ΔH (J/g) |
|--|--------------------|----------|
| Chitosan | 161.6 | 113 |
| Microspheres F1 without <i>E. palmifolia</i> | 167.7 | 61.6 |
| Microspheres F2 without <i>E. palmifolia</i> | 162.8 | 67.6 |
| Microspheres F3 without <i>E. palmifolia</i> | 158.3 | 101 |
| Microspheres F1 | 163.4 | 97.7 |
| Microspheres F2 | 162.1 | 95,2 |
| Microspheres F3 | 161.4 | 94.3 |

and 3358 cm⁻¹ (F2 and F3). There is no significant difference between the absorption of functional groups in the FTIR spectrum of chitosan, F1, F2, and F3 as shown in table 5, so it can be ascertained that the microsphere delivery system with Dayak onion extract as the active ingredient has been successfully formed.

X-Ray Diffraction (XRD) analysis: The result of the examination on *E. palmifolia* extract with X-Ray Diffraction (XRD) revealed that there was a most substantial intensity peak on the angle of 2θ as much as 15.809°, 16.776°, and 17.750°. Meanwhile, the result of examination on chitosan with X-Ray Diffraction (XRD) revealed that there was a sharp diffraction peak on the angle of 2θ at 19.86°, 37.72°, 43.96, 64.28° and 77.36°. The literature showed that chitosan has a weak diffraction peak at 11° and a sharp diffraction peak at 20° with X-ray source measurement conditions between 2θ that is around 10-30°.¹⁷

Differential Thermal Analysis (DTA): The examination of chitosan's melting point showed that chitosan had a melting point of 161°C with melting energy of 113 J/g. The literature stated that chitosan had a melting point of 150°C with melting energy of 122 J/g.¹⁸

The manufacture of microspheres delivery system for Dayak onion bulb extract in this study was carried out by making 3 formulas with different concentrations of chitosan in each formula. The concentration comparison between the active ingredient and chitosan in formula 1, formula 2 and formula 3 is 1:1, 1:2 and 1:3 respectively so that the concentration of the active ingredient used is 0.637% with variations in the concentration of chitosan at 0.637%, 1.275% and 1.912%. The purpose of doing variations in the concentration of chitosan in this study is to show the effect on the physical characteristics of the resulting microspheres. The method used in the manufacture of the microsphere delivery system is solvent evaporation. This method was chosen because it is easier to perform and does not require special instruments. In addition, this solvent evaporation method is able to produce a percent entrapment efficiency value that is almost 100%.

The formulation of this delivery system demonstrated the successful formation of microspheres. In this formulation, variations in the concentration of chitosan polymers (0.637%, 1.275% and 1.912%) were used which had an influence on the physical characteristics of the microspheres of the resulting Dayak onion bulb extract. The higher the concentration of chitosan used, the higher the entrapment efficiency and the yield will increase. Meanwhile, in observing the particle size, the higher the concentration of chitosan used, the smaller the particle size produced. The optimal concentration of chitosan as a polymer at concentration variations of 0.637%, 1.275% and 1.912% to provide good physical characteristics of the Dayak onion bulb extract

microspheres was shown in formula 3 with a chitosan concentration of 1.912%. The choice of the optimal concentration of chitosan as a polymer was due to the fact that at a chitosan concentration of 1.912%, the microspheres of the Dayak onion bulb extract were able to provide the highest percentage of entrapment efficiency and yield percentage that was almost 100%. In addition, at the chitosan concentration of 1.912%, it was able to produce microspheres of Dayak onion bulb extract in the form of a spherical shape with a flat surface and small particle size.

CONCLUSION

Variation of chitosan polymer concentration (0.637%, 1.275%, and 1.912%) affected the characteristics of microspheres of *E. palmifolia* extract preparation. The higher concentration of chitosan, the more the percentage of entrapment efficiency, and the percentage of yield also increased. Besides, the particle size decreased open the increasing chitosan concentration. The best physical characteristics of *E. palmifolia* extract microspheres were obtained from chitosan concentration at 1.912% (Formulation 3).

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GRAPHICAL ABSTRACT



Medicinal plant containing naphthoquinone, isoliquiritigenin, and oxyresveratrol



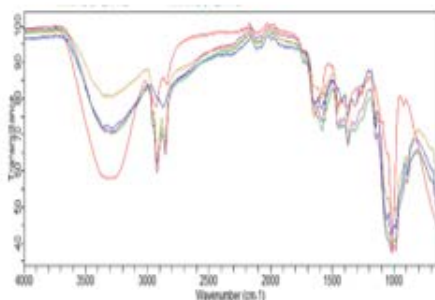
Formulate and characterize the *E.palmifolia* microspheres delivery system using chitosan variation as the



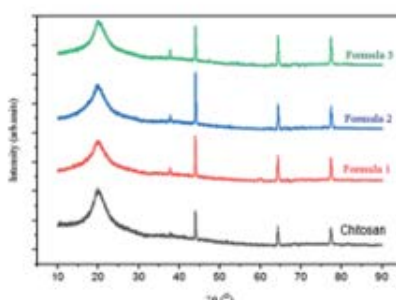
Preparation method was the solvent evaporation



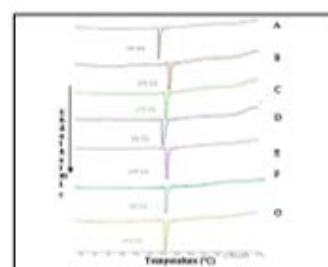
Results showed that chitosan concentration variation affected the microsphere's physical and chemical characteristics



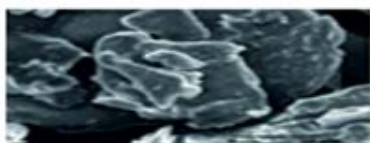
FTIR



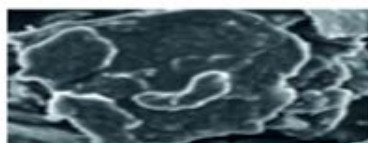
XRD



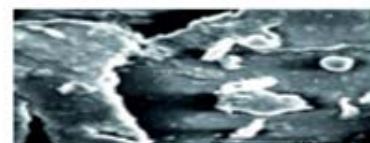
DTA



(A-F1)



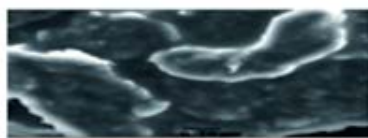
(B-F2)



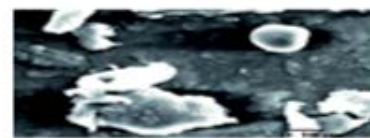
(C-F3)



(D-F1)



(E-F2)



(F-F3)

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