Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 10 No. 3 December 2023, 280-289
DOI: 10.20473/jfiki.v10i32023.280-289
Available online at https://e-journal.unair.ac.id/JFIKI/

Characterization of microencapsulated Saga Leaves Extract (*Abrus precatorius* L.) and Analgetic Activity Tests in Male Mice (*Mus musculus*)

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Submitted: 7 March 2023
Revised: 9 December 2023
Accepted: 13 December 2023

Abstract

***Background**: Saga leaves are one of the plants that have analgesic activity. Saga leaves contain phenol compounds, flavonoids, tannins, alkaloids, and saponins. Phenol has instability with oxygen, light, and high temperatures. Therefore, the microencapsulation process is necessary. Microcapsule characterization in this study included encapsulation efficiency, particle size, distribution value and morphology. **Objective**: This research was to determine the characterization of microcapsules and the analgesic activity of saga leaf extract microcapsules in male mice. **Methods**: The microencapsulation process conducted in this study was carried out using the spray drying method. Saga leaf extract was coated with the coating material in the ratio of 1:20. Several ratios of maltodextrin (MD) and soy protein isolate (SPI) (100%:0%); (75%%:25); (50%:50%); (25%:75%) were applied as the coating material. The encapsulation efficiency was determined by comparing the total phenol content of the extract and microcapsule. Particle size and distribution values were tested using a particle size analyzer. Microcapsule morphology was seen using scanning electron microscopy. Analgesic activity test using the tail-flick method with mice as test animals. Data analysis in this study used one-way ANOVA. **Results**: The encapsulation efficiency obtained was 31.40-80.29%. The particle size obtained in the microcosm was 17.70-30.90 µm. The distribution value obtained was 1.42-2.45. The morphology of the microcapsule obtained was round and had wrinkles. The analgesic activity obtained in this study resulted in significantly different pain inhibition values before and after microencapsulation. **Conclusion**: The characteristics of microcapsule preparations are well-known, and the analgesic activity of various microcapsules was 42.43-57.15%.*

***Keywords**: analgesic activity, microencapsulation, phenol, saga leaf extract*

How to cite this article:
Jehan, N. N., Sunarni, T. & Marlina, D. (2023). Characterization of microencapsulated Saga Leaves Extract (*Abrus precatorius* L.) and Analgetic Activity Tests in Male Mice (*Mus musculus*). *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 10(3), 280-289. http://doi.org/10.20473/jfiki.v10i32023.280-289

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

The saga plant grows wild in the forest or the yard of the house. Saga leaves have a sweet and bitter taste at the end when consumed. The bitter taste elicited from saga leaves is due to the presence of abrine (Agustina *et al.,* 2020). The content found in saga leaves, according to Nisak *et al*. (2021) are flavonoids, tannins, saponins, alkaloids and phenols. These phenolic compounds are unstable to oxygen, light or high temperatures, so their use will be a problem (Yang *et al*., 2016). Analgesic activity in research conducted by Indawati *et al*. (2020) with a dose of 110 mg/20 g BW of mice had a strength of 60.81% but was weaker than acetosal.

In recent years, drug development has been carried out in pharmaceutical preparations and technology for the manufacture of herbal-based drugs, one of which is microencapsulation. Saga leaves are herbal medicines that will be developed in microcapsule preparations because they have a bitter taste and retain the phenolic compounds contained therein (Agustina *et al.*, 2020) (Yang *et al.,* 2016). Siregar and Kristanti (2019) stated that microcapsules with a core and coating material ratio of 1:20 managed to coat the core material well compared to a 1:10 ratio. The content of total phenolic compounds from microcapsules resulted from a ratio of 1:20, which is greater than that of 1:10.

Maltodextrin is the most commonly used coating material in the microencapsulation process. Maltodextrin is obtained from hydrolysis of corn starch acid has a degree of dextrose equivalence between 10 to 20. Maltodextrin is also biodegradable and biocompatible, increases oxidation and stability, and prevents degradation (Wati *et al.,* 2020). In the world of food, soybeans are often used in intermediate products, namely protein isolates. Soy protein isolate coatings were used because the protein was a coating material that was capable of carrying active compounds and drugs so that it can optimize delivery on target (Dewandari *et al.,* 2013).

Preparation characterization is essential for microencapsulation to determine the quality of the preparation. Characterization of the microencapsulation includes particle size, distribution value (span), encapsulation efficiency of phenolic compounds and morphology. The particle size in microcapsules has a size range of 1-1000 µm. Encapsulation efficiency is a comparison between the total contents and the surface of the microcapsule. The distribution value (Span) is a measure of the width of the particle distribution to the microcapsules and aims to determine whether the particle distribution is uniform or not.

Nevertheless, scientific evidence regarding the microencapsulation of saga leaf extract has never been carried out. The results of this study will be able to provide information about the microencapsulation of saga leaf extract in relation to the analgesic activity of the ethanol extract of saga leaves. The aims of the study were to determine the characterization of microcapsules of ethanol extract of saga leaves from a combination of maltodextrin and soy protein isolate coatings using the spray drying method based on the test parameters of particle size, distribution value, encapsulation efficiency of phenolic compounds, morphology and to determine the analgesic activity in male mice.

MATERIALS AND METHODS

Materials

The materials to be used in this study were saga leaf Simplicia obtained at B2P2TOOT Tawangmangu, maltodextrin DE 10-12 (Chemipan Co., LTD), soy protein isolate (SPI), 70% ethanol (Merck), folin-ciocalteu, ibu profen, Na CMC, sodium acetate (Merck), distilled water, concentrated HCl, methanol (Merck), magnesium, mayer, wagner, dragendorf, $FeCl_3$ (Merck), Na_2CO_3 (Merck), $AlCl_3$, NaOH.

Tools

The tools used in this study were glassware (Pyrex), analytical balance (OHAUS Paj 1003), test tubes, rotary evaporator (BUCHI Rotavapor R-205, Germany), spray dryer (BUCHI B-290, BUCHI Labortechnik AG, Switzerland), PSA (Mastersizer 3000), SEM (FEI Quanta FEG 200 HR-SEM), UV-Vis Spectophotometry (Shimadzu UV-1800), vortex, stopwatch, oral3 syringe and tail flick analgesic meter.

Method

Extraction of Saga Leaf

Saga leaves that had been dried in the sun for three days (dry) were then crushed using a blender. Then, it was filtered using a 40 mesh sieve until powder was obtained. Saga leaf powder was macerated using 70% ethanol with a ratio of sample and solvent of 1:10 g/v for 6 hours with occasional stirring. The sample was allowed to stand for 18 hours, and then the filtrate was filtered. The remaining saga leaf pulp was remacerated using half the amount of the first solvent. The sample was again filtered using filter paper and evaporatedat a temperature of 40-50ºC (Indonesian Herbal Pharmacopeia, 2017).

Determination of water content

Weigh the sample as much as 10 grams and then put it into a dry flask. 100 mL of water-saturated Toluen was put into the flask, and the tool. Boil using low heat until

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E-ISSN: 2580-8303

no water drips; drain the water during the process. ed. The results of the water content obtained were recorded by looking at the volume on the scale of the tool. Moisture content is calculated in %b/v (Indonesian Herbal Pharmacopeia, 2017).

Determination of drying shrinkage

The determination of drying shrinkage was used using a moisture balance tool. We weighed a sample of saga leaf simplisia as much as 2 grams above the tool, then closed and waited until the % drying shrinkage appeared, which was marked by an alarm sound.

Phytochemical screening

Flavonoids

Take 0.1 g of concentrated extract of saga leaves into 2-3 mL of ethanol solution, heated, and enough magnesium powder and 2 mL of concentrated HCl were added. Colour changes to red, yellow, or orange indicate the presence of flavonoids (Hanani, 2021).

Alkaloid

Identification of alkaloids was carried out by adding mayer, dragendorff and wagner reagents. About 0.5 g of concentrated extract was added into 2 mL of concentrated HCl, then filtered. The filtrate obtained was divided into three parts then added mayer, dragendorff and wagner reagents (Hanani, 2021).

Tannins

A concentrated extract of 0.1 g was dissolved in 5 mL of hot water plus 2-3 drops of 5% FeCl solution. The presence of tannin content was indicated by a change in colour to blue or blackish green (Hanani, 2021).

Saponin

A total of 0.5 g of extract dissolved with 70% ethanol was added to 10 mL of warm water, and then shaken for 1 minute. Added HCl 2N as much as 2-3 drops. Observed the foam formed for 30 seconds (Hanani, 2021).

Phenol

Take 0.5 mL of the test solution with a concentration of 11 mg/mL and 0.5 mL of a gallic acid reference solution with a concentration of 150 µg/mL in a test tube, then add 2.5 mL of folin ciocalteu reagent. Dilute with distilled water at a ratio of 1/10 v/v in a test tube. Let stand for 10 minutes, add 7.5 mL of 1M sodium carbonate solution, and observe the colour of the solution with the naked eye (Rollando & Monica, 2018).

Encapsulation of saga leaf extract

Preparation of microcapsules using the ratio of core material: coating material, which is 1:20. Weighed 1 gram of extract and put it into a glass beaker. Maltodextrin, and soy protein isolate were added according to the formula weighing, and 79 mL of aquabidest was added, stirred until homogeneous with a magnetic stirrer at 700 rpm for 15 minutes. Put the preparation into a bottle and label it according to the formula. After that, the suspension was sprayed using a spray dryer with the temperature according to the formula for 1 hour. The microcapsule preparation was stored at room temperature for seven days.

Total phenolic analysis

The total phenolic content was analyzed with some modifications of the method listed in the Indonesian Herbal Pharmacopoeia (IHP) (Indonesian Herbal Pharmacopoeia, 2017). Make a 400 ppm gallic acid standard curve solution and then dilute it with a concentration series of 60, 50, 40, 30 and 20 ppm. Samples of 0.01 g extract and 0.21 g microcapsules were dissolved in 10 mL methanol p.a, homogenized with a vortex for 30 minutes and then filtered. Then 1 mL of sample was added with 5 mL of 7.5% folin ciocalteu and 4 mL of 1% sodium hydroxide. The solution was homogenized and incubated for 40-51 minutes in a dark room. Measurement of total phenolic absorption using a wavelength of 764 nm in UV-Vis spectrophotometry.

Total flavonoid analysis

The total flavonoid content was analyzed with some modifications of the methods listed in the IHP (Indonesian Herbal Pharmacopoeia, 2017). A 400 ppm quercetin standard curve solution was prepared and then diluted with a concentration series of 60, 50, 40, and 30 ppm. Samples of 0.02 g extract and 0.42 g microcapsules were dissolved in 10 mL ethanol p.a, homogenized with a vortex for 30 minutes, and then filtered. A sample of 0.5 mL was added to 1.5 mL ethanol p.a, 0.1 mL 10% aluminium chloride, 0.1 mL 1 M sodium acetate and 2.8 mL distilled water. The solution was homogenized and incubated for 16-32 minutes. Total flavonoid absorbance was measured using a wavelength of 441 nm in UV-Vis spectrophotometry.

Microencapsulation characterization

Encapsulation efficiency

Determination of encapsulation efficiency (%) was done according to Isailovic (2012). The encapsulation efficiency was calculated by comparing the total phenol content in the microcapsules with the extract.

$$\text{Encapsulation efficiency} = \frac{\text{total phenolic microcapsule}}{\text{total phenolic extract}} x\ 100\ \%$$

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E-ISSN: 2580-8303

Table 1. Saga leaf extract microcapsule preparation formula

Formula	Extract (g)	Maltodextrin (g)	Soy Protein Isolate (g)	Aquabidest (mL)	Inlet Temperature (°C)
F1	1	20	0	79	115
F2	1	5	15	79	115
F3	1	10	10	79	120
F4	1	15	5	79	125
F5	1	0	20	79	125

Particle size and distribution values

The particle size of the microcapsules was analyzed using a particle size analyzer (PSA) master-sizer 3000 laser diffraction (Malvern Instrument) carried out by dispersing the microcapsules with distilled water as much as 10 mL homogenized using a vortex mixer after homogeneous the sample was inserted into the cuvette in the tool until the appropriate intensity is reached and observed the analysis results (Siregar & Kristanti, 2019). Determination of the distribution value is obtained using the following formula:

$$\text{Span value} = \frac{D(90) - D(10)}{D(50)}$$

Description:
D(90) = Particle diameter at 90%
D(10) = Particle diameter at 10%
D(50) = Particle diameter at 50%

Morphology

Morphological analysis of microcapsules was determined according to Dewandri et al. (2013). The microcapsules obtained were analyzed for morphology using Scanning Electron Microscopy (SEM) Samples were placed in a sample holder and then coated with gold particles using a fine coater. Samples were analyzed and viewed morphologically at 1000x intensity and magnification.

Analgesic activity test

The test animals used were male mice aged 6-8 weeks, weighing 18-25 g. A total of 40 mice were randomly divided into eight treatment groups (each group of 5 mice). Group I mice (negative control) were treated with 1% CMC Na suspension orally. Group II mice (positive control) were given a suspension of 0.54 mg/20 g BW of ibu profen. Group III was treated with saga leaf extract 8 mg/20 g BW. Group IV, V, VI, VII, and VIII mice were each treated with saga leaf extract microcapsules 160 mg/20 g BW.

The analgesic test method used in this study was tail flick. The tail flicks analgesic meter will induce pain by irradiating the mice's tails with infrared at a wavelength of 70 foci. The nociceptive pain response was measured at 30, 60, 90 and 120 minutes.

Data analysis

The data obtained were analyzed in two ways: ANOVA analysis test and LSD analysis. ANOVA analysis is expressed as mean $\pm$ SD, where the test results are significant if $p \leqslant 0.05$. LSD further analysis was conducted with a 95% confidence level.

RESULTS AND DISCUSSION

Characteristics of saga leaf extract

In this study, saga leaf simplisia had a moisture content of 7.33% and a drying shrinkage of 11.67%. The water content of the simplisia is said to meet the requirements, but the drying shrinkage does not meet the criteria because it is more than 10%. This is likely to cause enzymatic processes and damage caused by microbes. The drying shrinkage of simplisia is more significant than the water content because the residual substances that evaporate during the shrinkage process are not only water but several compounds that can disappear at a temperature of 105°C. The research results of saga leaf extract are presented in Table 2.

Table 2. Characteristics of saga leaf extract

Characteristics	Value
Yield (%)	35.79
Water content (%)	3.33 ± 0.47
Total phenol (%)	6.70 ± 0.01
Total flavonoids (%)	3.66 ± 0.00

Note: average value based on three replications

The yield of ethanol extract of saga leaf obtained was 35.79%. The yield in this study is greater than the research by Nisak *et al*. (2021), which obtained a value of 31.4%. This is because this study used 70% ethanol solvent, which is more polar than 96% ethanol. So that, the yield ethanol extract of saga leaf obtained will be and more significant because the polar secondary metabolic will be more extracted. According to Situmeang (2019), the yield of saga leaf extract obtained was 10%. This happens because it uses a solvent with a low level of polarity so that it cannot attract polar compounds. The water content obtained in this study was 3.33% ± 0.470. This is in accordance with the requirements in FHI (Indonesian Herbal Pharmacopoeia) that the moisture

P-ISSN: 2406-9388
E-ISSN: 2580-8303

content of a good extract should not be more than 10%. The moisture content of saga leaf extract in research by Nisak *et al.* (2021) obtained a % moisture content of 2.83%.

Phytochemical screening

Phytochemical screening was carried out to determine the secondary metabolite compounds contained in the sample. Several kinds of secondary metabolites were tested, namely flavonoids, alkaloids, tannins, phenols, and saponins. These results have similarities with the results obtained in a study by Nisak *et al.* (2021). The results of the phytochemical screening of saga leaf extract are presented in Table 3. The table shows that all tests carried out are positive.

Total phenol and flavonoid content in extracts

Determination of phenolic content was carried out with folin-ciocalteu reagent, which will oxidize the hydroxyl group of phenol group compounds to produce complex compounds marked by the colour of the yellow solution turning blue-green. The process runs slowly in an acidic atmosphere, so in the test, sodium hydroxide is added, which is alkaline, so as to make the reaction faster. The standard solution used was gallic acid, which was one of the stable phenolics and was classified as a simple phenol group capable of high reactivity to folin-ciocalteu. The maximum wavelength was obtained at 764 nm, and the operating time was 40-51 minutes. The correlation coefficient value obtained was 0.9977, and the linear regression equation for gallic acid absorbance obtained y = 0.010x + 0.1143, which was used to determine the total phenolic content in extracts and microcapsules of saga leaf extract.

Flavonoids are natural phenol class compounds that are distributed in various plants. The determination of total flavonoids in extracts and microcapsules of saga leaf extract used UV-Vis spectrophotometry with a maximum wavelength of 441 nm and an operating time of 16-32 minutes. The reagent used in the determination of total flavonoids was aluminium chloride, which was often referred to as the colourimetric method. This method is a very simple method for testing flavonoids of essential flavone and flavonol groups so that they react with Al (III) to turn the sample into a yellow color. The linear regression equation obtained is y = 0.0098 and R^2 value = 0.9954. Table 2 states that the phenol and flavonoid content in saga leaf extract is 6.70% and 3.66%, respectively.

The high phenol content can be caused by several factors, one of which is the use of solvents during the extraction process. The solvent used in the extraction is ethanol, which has polar properties that can attract polar compounds such as phenols, flavonoids, tannins, steroids, and alkaloids (Dia *et al.*, 2015). Aside from the use of proper solvents, the mature saga leaf sample is what causes the high amounts of a sample. As a result, the content of secondary metabolites is larger in the older ones. Anwar *et al*. (2017) discovered that older leaves had greater phenolic levels when comparing phenolic levels based on leaf age in Aquilaria beccariana plants.

Characterization of saga leaf extract microcapsules

Total phenol and flavonoid content in microcapsules

From Table 4, the results of the total phenolic content in the extract and microcapsule preparations produced higher levels. This could occur due to the influence of the temperature used during the microencapsulation process using spray drying. In line with research by Kistriyani *et al.* (2020), the total phenolic content in microcapsules decreased after encapsulation, where the phenolic content in the extract was 0.34 g/mL, while in microcapsules, the complete phenolic content was 0.09 g/mL. In microcapsules, F1 is lower than F2; this is because soy protein isolate has a high viscosity, so it allows the homogenization process between the extract and the dressing to be uneven. So, the sample content taken has a trim level. The combination of maltodextrin is essential to homogenize the preparation because it has a lower viscosity so the highest level is obtained in F2.

Table 3. Phytochemical screening results of saga leaf extract

Phytochemical Test	Reagent	Results
Flavonoid	Magnesium dan HCl Concentrated	Yellow (+)
Alkaloid		
	a. Mayer	a. Beige precipitate (+)
	b. Wagner	b. Brown precipitate (+)
	c. Dragendroff	c. Orange precipitate (+)
Tanin	$FeCl_3$	Blackish Green (+)
Phenol	Folin-ciocalteu dan Na_2CO_3	Purple-black (+)
Saponin	HCl 2N	Foam stabil (+)

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E-ISSN: 2580-8303

Table 4. Characterization of saga leaf extract microcapsules

Sample	Total phenol (%)	Total flavonoids (%)	Encapsulation efficiency of total phenol (%)	Particle size (µm)	Distribution value
F1	5.15 ± 0.01	3.20 ± 0.03	76.89 ± 0.26	30.17 ± 0.57	2.36±0.08
F2	5.38 ± 0.00	3.20 ± 0.01	80.29 ± 0.27	25.23 ± 0.57	1.63±0.02
F3	4.83 ± 0.02	2.22 ± 0.00	72.16 ± 0.33	23.60 ± 0.37	1.77±0.03
F4	2.80 ± 0.01	1.70 ± 0.00	41.75 ± 0.14	17.87 ± 0.04	1.42±0.01
F5	2.10 ± 0.01	1.53 ± 0.00	31.40 ± 0.12	-	-

The microcapsule F3 formula obtained a viscosity that was not too high, but in this preparation, a temperature of 120oC was used to produce a preparation that was dry enough. As for microcapsules F4 and F5 using a temperature of 125ºC, this is because the nature of maltodextrin has high hygroscopicity, causing the ability to bind saga leaf extract to decrease and requires a high temperature during the spray drying process to produce a preparation that is dry enough. The influence of higher temperatures will affect the degradation of polyphenols and the release of phenolic components such as hydroxyl groups, resulting in a decrease in phenolic levels. Phenolic compounds are thermolabile and oxidative compounds, so they can influence the in the reduction of phenolic levels at high temperatures. The temperature in the encapsulation process can hydrolyze polyphenolic compounds into simpler phenolic compounds (Mahardani and Yuanita, 2021).

The flavonoid content produced in microcapsules of saga leaf extract is 3.20% ± 0.01 - 1.53% ± 0.00. The levels obtained are lower than the flavonoid levels in the extract. This is in line with the results obtained in the measurement of phenolic compounds. Flavonoids degrade when the temperature is above 90ºC. Flavonoids are heat sensitive due to their hydroxyl and ketone groups, as well as unsaturated double bonds (Qiao *et al.,* 2014). The concentration of phenol and flavonoid totals in the F2 formula has the highest level; this is in line with research by Sadiah *et al.* (2022) that treatment with a high concentration of soy protein isolate produced higher flavonoid levels than the treatment with a high concentration of maltodextrin. Soy protein isolate has good protection against oxidation. However, the use of maltodextrin combined with soy protein isolate aims to produce an amorphous glass matrix that acts as a barrier to the oxidation process during the encapsulation process (Sadiah *et al.,* 2022). Total flavonoids of 3.20% and total phenols of 5.38% in the F2 formula were the highest values in this study.

Encapsulation efficiency of total phenol

The encapsulation efficiency of total phenol was a determination that aims to determine how many active compounds are trapped in the encapsulation process and determine the success rate of the encapsulation process. The results of the encapsulation efficiency are in Table 4. From the analysis, it was found that each group of microcapsules had significant differences in encapsulation efficiency. The most excellent encapsulation efficiency in this study was obtained in the F2 microcapsule formula and the most negligible efficiency in the F5 formula. Several factors influence encapsulation efficiency. Specifically, the coating material utilized, as well as the ratio of active ingredient and coating material temperature during encapsulation, as well as the viscosity drying rate and surface cracks. The combination of maltodextrin coating material and soy protein isolate was effectively used in research conducted by Sadiah et al. (2022) with the encapsulation method using a spray dryer. The ratio of the active compound and the coating material in this study used a ratio of 1:20, which is in line with research conducted by Siregar and Kristanti (2019), which states that with this ratio, the results obtained are better microcapsule characteristics, including encapsulation efficiency and total phenol than used comparison with the ratio of the active compound and the dressing material 1:10.

Particle size and distribution value

The results of the data analysis obtained said that there were significant differences in particle size and distribution value of each group of microcapsule formula. It can be concluded that each combination concentration can affect the results of particle size and distribution value. The results of the particle size and distribution value are in Table 4. The results of the particle size obtained: the greater the concentration of soy protein isolate the greater the particle size produced. This is because the viscosity of the suspension is higher than the concentration of soy protein isolate. This is due to the higher viscosity of the suspension compared to the maltodextrin treatment with more concentration. The higher the viscosity, the larger the droplets formed during the encapsulation process and the larger the particle size. In line with research by Metaviani *et al*. (2013), the particle size obtained gets more extensive if the concentration of maltodextrin gets smaller. The particle size values obtained in this study ranged from

P-ISSN: 2406-9388
E-ISSN: 2580-8303

17-30 µm. This is in line with what was stated by Hidayah (2016) that the particle size produced using a spray dryer is in the range of 10-400 µm.

The distribution value is set to determine the distribution of particle sizes produced during the encapsulation process. The distribution value is obtained from the particle size value at 90% minus the particle size value at 10% divided by the particle size value at 50%. This happens because there are differences in the concentration of the coating material or perhaps when the formula loading process is not evenly distributed, resulting in a very high distribution value. The distribution value of microcapsules with concentration F4 was found to be the best value. This value indicates that the presence of maltodextrin plays a role in the uniformity of particle distribution. This is because, theoretically, the smaller the distribution value. The better the uniform particle size distribution (Krishnaiah *et al.*, 2012). Because the preparation was water soluble, the distribution value for microcapsule F5 could not be read.

Morphology test

From the results obtained, it can be seen that the shape of the microcapsules is round. There are clumps that are not uniform and irregular. This is in line with research conducted by Krishnaiah *et al.* (2012), most particles have a round shape and show little agglomeration. There are differences in the morphology of F1 and F2 microcapsules with other formulas. The results of F1 microcapsules have a larger size and are more heterogeneous. In the morphology of microcapsules with F1 concentration, it can be seen that smaller particles are attracted to and attached to larger particles around them. This happens because of the heterogeneity, which makes the force of attraction and clumping between the particles stronger (Sari, 2021).

From Figure 1, the results obtained show that the shape of the microcapsules is round, and some clumps are not uniform and irregular. This is in line with research conducted by Krishnaiah *et al.* (2012), most particles have a round shape and show little agglomeration. There are differences in the morphology of F1 and F2 microcapsules with other formulas. The results of F1 microcapsules have a larger size and are more heterogeneous. In the morphology of microcapsules with F1 concentration, it can be seen that smaller particles are attracted to and attached to larger particles around them. This happens because of the heterogeneity of the particles, which makes the force of attraction and clumping between the particles stronger (Sari, 2021).

The suspension between the active compound and the coating in this study used aquabidest so that the interaction between maltodextrin and aquabidest causes the hydroxyl groups contained therein to dissolve (Yuliawaty & Susanto, 2015). DE describes Maltodextrin (dextrose Eqipvalent), where the higher the DE value, the higher the water solubility, so this is related to the microcapsule morphology. The maltodextrin used in this study has a DE value of 10-11, which is high, so preparations with high maltodextrin concentrations will cause the preparation to be hygroscopic. From this, it is important to combine with soy protein isolate to produce preparations with good morphology.

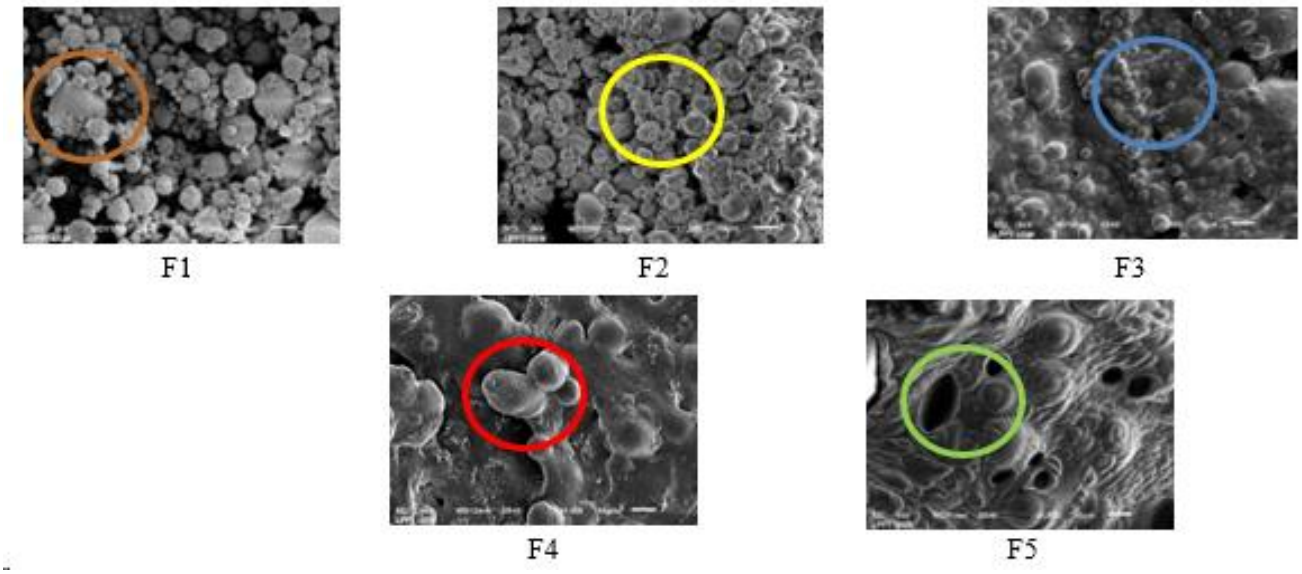

Figure 1. Surface morphology of saga leaf extract microcapsules of all groups with SEM

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Analgesic activity test

The tail flick method was used to investigate analgesic activity in eight different test groups. This study was approved by the ethics commission of health research at Dr. Moewardi Hospital with letter number 1.369/X/HREC/2022. In Figure 2 ΔT1, the results of the analysis of the extract group and microcapsule preparations showed significant differences with the positive control. In the F2 microcapsules group, there were significant differences in the negative, positive and extract groups. The F4 microcapsule group only had a significant difference in the positive and extract groups in ΔT2, and in ΔT3 there was only a significant difference in the negative and extract groups. In ΔT4 groups F1, F2, F3, and F4, there were significant differences between the negative, positive, and extract control groups. Meanwhile, in the F5 microcapsule group, there were no significant differences from the negative, positive, and extract control groups. In Figure 2, it was obtained that the positive control group has an inhibitory response that is significantly different from the negative control group. This happens because ibuprofen has not given a maximum response because the absorption of ibuprofen in blood plasma takes 30-60 minutes and will reach onset after one hour, so that the response that appears is not significantly different compared to saga leaf extract preparations tested. Saga leaf extract gives a response that is not significantly different from ibuprofen, this is because the ethanol extract of saga leaves contains flavonoids, terpenoids and other secondary metabolites that function as analgetics. However, the microcapsule preparation is significantly different from ibuprofen at ΔT2, this is because the microcapsule preparation has a coating which results in the release time of the active substance requiring a longer time. The microcapsule preparation reaches the highest pain inhibition time at 90 minutes. The microcapsule preparation will pass through the human digestive tract so that the outer layer of the microcapsule will dissolve in the stomach due to the lower pH atmosphere, so that only the core material is absorbed in the small intestine. The length of the active substance release process is due to the content of the coating more than the active substance. The greater the active substance content. The process of releasing the active substance from microparticles increases more (Sinha *et al*.. 2004).

The data Increase in pain inhibition was continued with the one-way ANOVA test and the results of the analysis in the positive control group were significantly different compared to the other groups because the sig value <0.05. The extract and microcapsule groups all have significant differences because they get a sig value <0.05. In the F1 microcapsule group, there is no significant difference from the F2 and F3 groups because it gets a sig value >0.05. In microcapsules F4 and F5, there is no significant difference in the value of increasing pain inhibition. Based on Table 5. the results of the acquisition of pain inhibition produced by the extract group. F1. F2 and F3 microcapsules are more than 50%. according to Puspitasari *et al*. (2003). it was stated that they had analgesic activity. Th,e content of secondary metabolite compounds such as flavonoids present in the sample can influence this, which are thought to have analgesic activity with a mechanism of action capable of inhibiting the cyclooxygenase pathway that produces pain mediators such as prostaglandins, histamine,bradykinin and leukotrien. The presence of flavonoids can also affect the metabolic activity of arachidonic acid enzymes. The higher the flavonoid content, the higher the arachidonic acid content that can inhibit cyclooxygenase. resulting in high analgesic power.

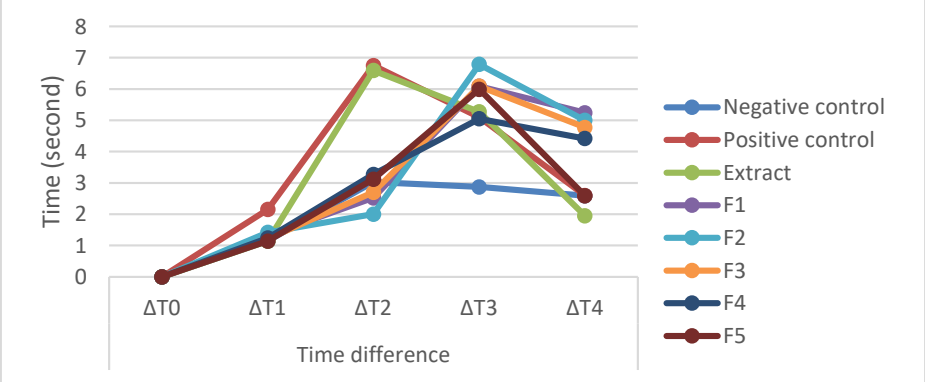

Figure 2. Data graph of the average difference in pain inhibition response time

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 5. AUC data and percentage of pain inhibition

Sample	AUC data (Total ± SD)	Increase in pain inhibition (%) (Mean ± SD)
Negative Control	242.67 ± 6.05	-
Positive Control	458.67 ± 11.66	89.01 ± 1.62
Extract 8mg/20gr	418.92 ± 8.47	72.67 ± 2.82
F1 160 mg/20gr	376.44 ± 8.51	55.25 ± 5.96
F2 160 mg/20gr	381.33 ± 10.11	57.15 ± 2.15
F3 160 mg/20gr	371.52 ± 9.16	53.15 ± 4.12
F4 160 mg/20gr	352.98 ± 13.04	45.48 ± 4.95
F5 160 mg/20gr	345.66 ± 15.72	42.43 ± 5.06

CONCLUSION

The results of microencapsulation characterization of saga leaf extract from a combination of maltodextrin and soy protein isolate based on particle size parameters, distribution values, phenolic compound encapsulation efficiency and morphology by spray drying method each obtained an average value of 17.70-30.90 µm; 1.42-2.45; 31.40-80.29% and round shape and has wrinkles, respectively. The analgesic activity obtained in this study resulted in significantly different pain inhibition values before and after microencapsulation. The analgesic activity of various microcapsules was 42.43-57.15%.

SUGGESTION

The results of the high distribution value in microencapsulation of saga leaf extract are too high so it needs to be improved in terms of manufacturing and the ratio of coating materials for further research.

ACKNOWLEDGMENT

The author would like to thank STIKES An Nasher Cirebon for funding the Masters program.

AUTHOR CONTRIBUTIONS

Conceptualization, N. N. J., T. S., D. M.; Methodology, N. N. J., T. S., D. M.; Software, N. N. J.; Validation, N. N. J.; Formal Analysis, N. N. J., T. S., D. M.; Investigation, N. N. J.; Data Curation, N. N. J., T. S., D. M.; Writing - Original Draft, N. N. J.; Writing - Review & Editing, T. S.; Visualization, D. M.; Supervision, T. S., D. M.; Project Administration, N. N. J.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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P-ISSN: 2406-9388
E-ISSN: 2580-8303