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Encapsulation of Ethanol Extract of Jeruju (*Acanthus ilicifolius* L.) Leaves and It's Antiinflammatory Activities Against Carrageenan-Induced Mice

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ABSTRACT. Jeruju (*Acanthus ilicifolius*) leaves ethanol extract contains several bioactive compounds that might function as an antiinflammatory agent. Coating technology through the encapsulation of bioactive compounds is necessary and much research has been carried out to increase the utilization of plant extracts as medicinal preparations or supplements. The specific aim of this study was to observe changes in the anti-inflammatory activity of the extract and encapsulated preparations of the ethanol extract of jeruju leaves as anti-inflammatory agents using mice. The research method begins with preparing samples to be extracted with ethanol solvent, phytochemical screening tests, extract encapsulated formulations, percent yield and solubility of the encapsulated extract, and anti-inflammatory tests. This research used maltodextrin coating material for encapsulated, carried out using the freeze-dryer method, and and using SEM and FTIR to analyze the results of the encapsulated extract. The anti-inflammatory test was carried out on 60 male white mice aged 2-3 months with a body weight of approximately 15-30 grams and the test was carried out using the rat hind paw edema method or the formation of artificial inflammation on the soles of the mice's left paws using carrageenan. Edema volume was measured using a plethismometer every 60 minutes for 330 minutes. This research shows that the secondary metabolite compounds contained in jeruju leaves ethanol extract are flavonoids, phenolics, steroids and saponins. Several extract encapsulation formulations obtained the highest yield of 58.81%, and the highest solubility of 98.76 The average percent inhibition of each dose administered is calculated based on the data results and the high average percent inhibition obtained for Na–diclofenac is 80%. In the research encapsule 1:12, the 120 mg/KgBW dose had a significant percentage of inhibition of 75.55%, with Na–diclofenac dose of 10 mg/KgBW being 80%. This study highlights the potential of jeruju leaf extract as an effective anti-inflammatory agent when encapsulated, presenting a promising avenue for further research and applications in medicinal contexts.

Keywords: Antiinflammatory, encapsulation, jeruju (*Acanthus ilicifolius* L.)

INTRODUCTION

Traditionally the Jeruju plant (*Acanthus ilicifolius*) is used as an asthma remedy (fruit); diabetes, diuretic, hepatitis, leprosy (fruit, leaves, and roots); neuralgia, roundworm, rheumatism, skin diseases, abdominal pain (bark of stems, fruits, and leaves), antifertility, skin diseases, tumours, and ulcers (resin) (Bandaranayake, 2002). Decoction of *Acanthus ilicifolius* (*A. ilicifolius*) leaves most widely used for restoring energy after childbirth by boiling and used for bath water, restoring stamina after childbirth, and preventing infection in the uterus (Suryati et al., 2018). *A. ilicifolius* plants contain glycoside compounds, alkaloids, flavonoids, fatty acids, steroids, lignans, phenol, and terpenoids components (Poorna et al., 2011). Inflammation is a local protective response caused by damage to tissue caused by physical trauma, damaging chemicals, or microbiological substances. Inflammation destroys, reduces, or localizes (sequesters) both damaging agents and tissues. Signs of inflammation are swelling or oedema, redness, heat, pain, and changes in function. Drugs that can inhibit the release of inflammatory mediators that cause inflammatory reactions in heat, pain, swelling, red, and impaired function are called anti-inflammatory. The definition of anti-inflammatory is also a class of drugs that suppresses or reduces inflammation (Peres et al., 2012). Concentrated dye extracts are often unstable, so they need to be converted into solid form, and one of the techniques that can be done is encapsulation (Chairany et al., 2021).

Encapsulation is one method to facilitate handling, improve solubility, and stability, protect against toxicity, increase pharmacological activity, slow-release, and protect against physical and chemical degradation. Encapsulation aims to protect sensitive material components and reduce the degradation of active compounds in materials (Zabot et al., 2022). Encapsulation technology, in addition to maintaining

bioactive components, can also maintain flavor, especially in essential oils or oleoresins. Bioactive compounds and oleoresin components are trapped in a polymer coating, forming spherical microcapsules with sizes of tens of microns to a few millimeters (Mahmoud et al., 2018). Several coating materials are used in encapsulation, one of which is maltodextrin. Maltodextrin is a natural polymer from hydrolysis α-amylase with a DE (Dextrose Equivalent) value of less than 20 (Hofman et al., 2016). The advantages of maltodextrin are that it has low viscosity in many ratios, high solubility, no taste is economical, has low browning properties, and has solid binding power (Wei & Sulaiman, 2022). This research aimed to increase the use of ethanol extract of *A. ilicifolius* leaves in the form of an encapsulated formulation as an anti-inflammatory.

EXPERIMENTAL SECTION

Materials and Methods

The primary material used in this research is Jeruju Leaves (*Acanthus ilicifolius* L.), which obtained from East Tanjung Jabung Regency, Jambi Provinces. Some chemicals was used; Ethanol, Maltodextrin, Aquadest, carrageenan, 0.9% physiological NaCl, Diclofenac-Na, CMC-Na, Tween-80, Reagent dragendorff, Mayer reagent, Mg powder, 2N HCl, concentrated HCl, $FeCl_3$, concentrated sulfuric acid, anhydrous acetic acid (Sigma Aldrich). The instrument that used to support this research, rotary evaporator (R-100 with Cold Trap Buchi), magnetic stirrer, Plethysmometer (Orchid Scientific), and freeze dryer (Christ Alpha).

Extract content analysis was determined using a UV-Vis spectrophotometer (Thermo Fisher Scientific), and the determination of encapsulation characteristics was analyzed based on FTIR (Thermo Fisher Scientific), and SEM (Hitachi SU-3500) results. FTIR analysis was performed at wave numbers 400-4000 cm-1. Furthermore, SEM analysis was performed to determine the shape and morphology of the encapsulation obtained from the encapsulation formulation with a magnification of 500×; 1000×; 2500× and 5000×.

Animals

The test for anti-inflammatory activity used in this research was mice (Mouse muscle) from the Department of Biology, Faculty of Science and Technology, Universitas Jambi. In vivo studies of anti-inflammatory activity were conducted using 60 male mice aged 2-3 months with a body weight of 15-30 grams that had been quarantined to adapt to their environment for one week. Animal experiments comply with the Care and Use of Animals Indonesian guidelines and approved by the Health Research Ethics Commission from the UNJA Faculty of Medicine and Health Sciences No. 1397/UN21.8/PT.01.04/2023.

Sample Preparation and Extraction

A. ilicifolius leaves were taken from East Tanjung Jabung Regency, Jambi Province, Indonesia. The leaves are washed, dried, and crushed to obtain leaves powder (simplicia). The simplicial was macerated for 4-5 days in 70% ethanol solvent. Then, filtered macerate using a funnel and paper Whatman. The filtrate was evaporated using a rotary evaporator at a temperature of 68 °C until concentrated. The weight percentage was calculated between the yield and the weight of the simplicia powder used by weighing.

Phytochemical Screening

Alkaloids test

As much as 1 mL of the sample was dissolved in a few drops of 2N sulfuric acid and then tested with three alkaloid reagents: Dragendorff reagent, Mayer, and Wagner's. The test result was positive if the Dragendorff reagent formed a red-to-orange precipitate, the Meyer reagent formed a yellowish-white precipitate, and the Wagner reagent formed a brown precipitate (Latief et al., 2023)

Flavonoids test

Then, a few drops of concentrated HCl were added to several samples, and Mg powder was added. Positive results from the HCl reagent and Mg powder were indicated by the formation of foam and a change in the colour of the solution to orange (Latief et al., 2023).

Saponin test

The foam test in hot water can detect saponins. Stable foam that can last a long time and does not disappear with the addition of 1 drop of 2N HCl indicates the presence of saponins (Latief et al., 2023).

Tanin test

Several samples were added to FeCl3 then the mixture was homogenized. A positive reaction is indicated by the formation of a greenish-black colour in the mixture (Latief et al., 2023).

Steroids and triterpenoids test

Several samples were added with anhydrous acetic acid and concentrated sulfuric acid (Liebermann-Burchard). When a blue or green colour is formed, it indicates the presence of steroids. When a purple or orange colour is formed, it indicates the presence of triterpenoids (Latief et al., 2023).

Preparation of test solutions

Take 10.00 mg of condensed extract and add ethanol up to 10 mL to obtain a test solution of 1000 ppm. 1 mL of the 1000 ppm test solution was taken and then reacted with the same procedure as the standard extract solution.

Encapsulation Formulation

The encapsulation process of ethanol extract from *A. ilicifolius* leaves refers to previous study (Pudziuvelyte et al., 2020). The emulsion is formed from a coating material, namely maltodextrin, which will be dissolved in water, and the main ingredient is added, the ethanol extract of Jeruju leaves. With several variations, the ratio between the core material and the encapsulant material is 1:8; 1:10; 1:12; 1:14,

and 1:16. The ingredients are then mixed in a stirrer for 1 hour using a stirrer magnetic with a stirrer speed of 800rpm. Added 1 mL of tween 80 as an emulsifier. Then homogenized for 20 minutes using a homogenizer. Next, the drying process is carried out. The freeze dryer is at -55 °C to obtain encapsulated powder of ethanol extract of *A. ilicifolius* leaves.

Physical and Chemical Properties of Encapsulation

Percent product yield

The yield calculation is done after it is obtained encapsulated Jeruju leaves extract. The yield obtained from the initial weight after and before drying using freeze drying.

Encapsulation solubility in water

Solubility was determined, referring to the method used by previous study (Wardhani et al., 2020) with some modifications. 0.1 g of encapsulation was added to 10 mL of water at 30 °C and stirred using a magnetic stirrer. Then the solution is filtered with filter paper Whatman, whose fixed weight is known. The filter paper and the unfiltered part of the sample were put into the oven for 1 hour at 105 °C, then cooled in a desiccator for 15 minutes, and then weighed. The unfiltered sample weight was obtained from the difference between the final and initial filter paperweights.

Antiinflammatory Activity Testing

Extract suspension preparation

Jeruju leaves ethanol extract and encapsulation were suspended with diclofenac Na in distilled water. CMC Na was sprinkled over hot water in a mortar using water as much as 20 times the weight of Na CMC and left for 15 minutes until the Na CMC swelled. Then the extract was added gradually into the mortar while homogeneously crushed and filled with equates up to 10 mL.

Preparation of 1% carrageenan solution

A 1% carrageenan solution was prepared by dissolving 1 gram of carrageenan in 100 mL of 0.9% physiological NaCl solution.

Antiinflammatory activity test

Antiinflammatory activity test was carried out on positive control (Diclofenac Na), negative control (CMC Na), ethanol extract of Jeruju leaves, and encapsulation of ethanol extract of Jeruju leaves. Before testing, mice were fasted for 18 hours (no food but still given water). Each group consisted of 3 mice. Categories of each group, namely;

C0 = negative control (-) for 1% Na CMC;
C1 = control (+) for 10 mg diclofenac Na;
C2 = ethanol extract of jeruju leaves 300 mg/kg BW;
C3 = ethanol extract of jeruju leaves 600 mg/kg BW;
C3 = ethanol extract of jeruju leaves 900 mg/kgBW.

Meanwhile, the encapsulation antiinflammatory activity was evaluated with three dose variants, namely 60, 90, and 120 mg/KgBW.

Each animal was weighed and marked on the tail during testing. After that, the left leg of the mouse was put inside Plethismometer, and then the volume was recorded as the initial volume (Vo), which is the volume of the leg before being given the drug. Each foot of the mice was injected subplantar with the solution carrageenan 1% as much as 0.1 mL. After thirty minutes, measurements were taken by dipping the mice's feet into the tube plethysmometer. According to the group, each mouse was given a suspension of the test material orally. Changes in the fluid volume are recorded as the volume of the soles of the mice's feet at each observation time (Vt). Measurements are taken every 60 minutes for 300 minutes (Hasanah & Hidayah, 2018). After measurement, the volume of mice foot oedema was calculated, AUC (Area Under the Curve) from the average oedema curve against time and percent inflammation inhibition (Aprilianto et al., 2019). The data were then tested with One-Way ANOVA and Post Hoc LSD test with a 95% confidence level by using SPSS 25 software.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening was carried out to provide an overview of the class of compounds contained in the plants studied. The phytochemical screening method tests the colour using a colour reagent. The results of the phytochemical screening of the ethanol extract of Jeruju leaves can be seen in **Table 1. Table 1** shows that the ethanol extract of Jeruju leaves in this study did not contain alkaloid compounds, presumably because the extraction process may be inefficient in isolating the alkaloids from the sample, resulting in lower alkaloid yields in ethanol solvents, the temperature was too high and the difference in polarity between the alkaloid compounds and the solvent a high temperature can lead to chemical changes in the compounds being extracted, potentially reducing the quality of the extract.

Additionally, if the polarity of the solvent doesn't align well with the alkaloid compounds, it can result in inefficient extraction, meaning that not all of the desired compounds may be effectively extracted. These factors are critical considerations in optimizing extraction processes. In addition, the difference with the results of previous studies which had an alkaloid component was suspected that the difference in sample location affected the bioactive component, so that in this study phytochemical skinning was carried out to confirm the components present, which may be different from previous studies (Kaboré et al., 2022).

Physico Chemical Properties

Percent yield of encapsulated products

The yield is the ratio of the weight of the encapsulation obtained to the weight of the raw material. The encapsulation obtained from the drying results is

calculated to determine the percentage of yield by weighing the weight of the obtained encapsulation and then dividing it by the weight of the emulsion before drying. The yield value is related to the amount of bioactive content contained in Jeruju leaves (Ananda et al., 2022). The higher the yield value, the more encapsulation values are produced. Yield is calculated in the form of percent (%). The data on the proportion of yield of encapsulated products can be seen in **Figure 1.** Based on **Figure 1**, this indicates that the greater the percentage of maltodextrin coating, the greater the percentage of product yield produced. This is to the results of research by Djafar & Supardan (2019) namely, the greater the concentration of maltodextrin in the ginger oil used, the greater the yield of ginger essential oil microcapsule products obtained. According to Yuliawaty & Susanto, (2015) the increase in yield is influenced by a large amount of maltodextrin added because the more maltodextrin, the greater the total solids obtained. One of the functions of using maltodextrin in certain products is as a filler which can increase the yield of the resulting final product (Putri et al., 2019).

Solubility is a parameter that indicates the ability of a powder to dissolve again in water. The powder is a food and beverage shield requiring a high solubility value. The greater the solubility value of a powder, the more quickly it will dissolve in water. The data from **Figure 2** and the test results above show that the encapsulated ethanol extract of Jeruju leaves dissolves easily in water. The solubility of encapsulated products in water is classified as high, ranging from 94.44% - 98.76%. Where the more maltodextrin added, the more excellent the solubility. The high solubility of maltodextrin will increase the encapsulation solubility. The excellent solubility properties of the product in powder form are 95% (Ningsih et al., 2019). Meanwhile, the encapsulation of the ethanol extract of Jeruju leaves has a solubility of 95.44% - 98.76%. Thus, the encapsulation of the ethanol extract of Jeruju leaves can be said to be a product in powder form, which has good solubility. So from the fifth formulation tested, the 1:16 encapsulation formulation showed the best solubility of 98.76%. The higher the concentration of maltodextrin, the faster the solubility because maltodextrin can increase the total solids so that the water content decreases.

Tabel 1. Phytochemical screening of ethanol extract

Secondary Metabolites	Result
Alkaloids	
Meyer	-
Dragendorff	-
Flavonoids	+
Phenolics	+
Steroids	+
Saponins	+

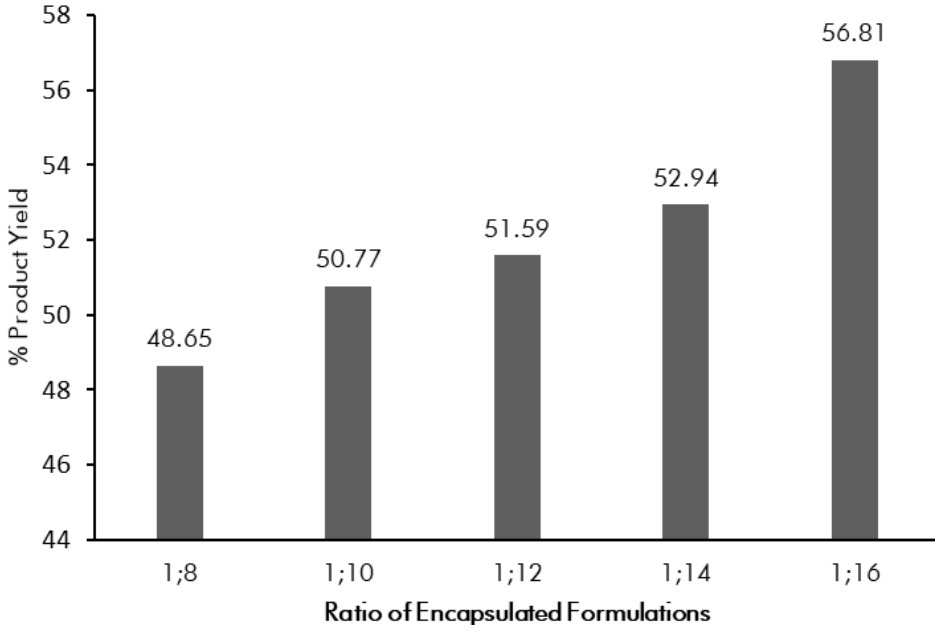

Figure 1. The yield of encapsulated products

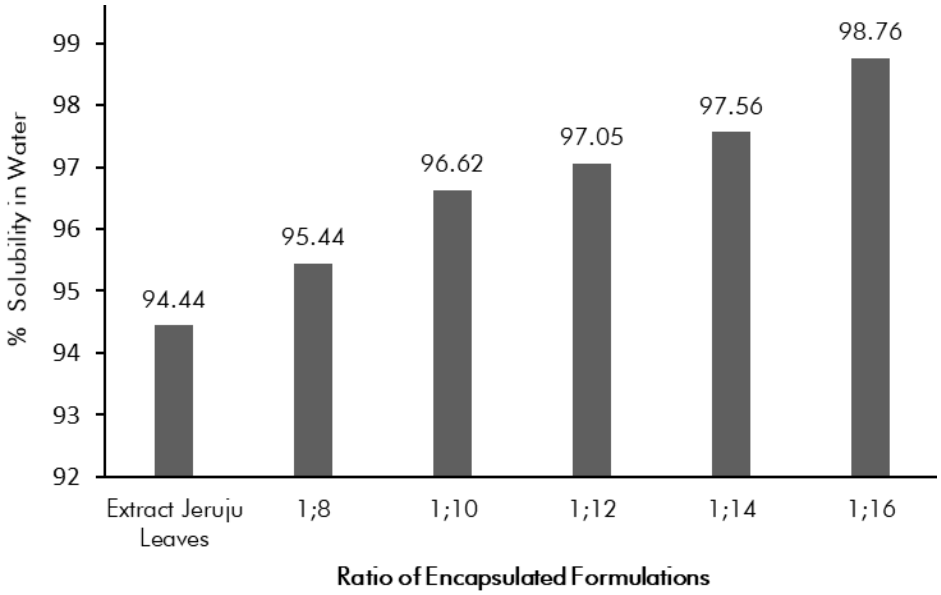

Figure 2. Solubility of encapsulations in water

According to previous studies, the higher the maltodextrin concentration, the higher the solubility value (Ningsih et al., 2019). This happens because maltodextrin has the property of being able to bind hydrophobic substances. Besides, maltodextrin is a polysaccharide soluble in water to form a uniformly dispersed solution. According to Yuliawaty and Susanto, (2015), the hydroxyl groups in maltodextrin will interact with water when the material is dissolved. The added maltodextrin can bind water in the material and maintain the water content so that a large amount of maltodextrin added will affect the yield. The increase in yield, along with the proportion of maltodextrin, indicates that maltodextrin has a better ability to coat extracts, in this case, the ability to form emulsions, film formation, and flexibility in coating extract FT-IR Spectroscopy

The infrared spectrum of each sample was recorded in the FTIR 640-IR variant spectrophotometer with the wavenumber range used 4000-500 cm^{-1}. The FTIR characterization results obtained are shown in the following **Figure 3**. The chemical interaction of Tween 80, Maltodextrin, and extract were investigated using FT-IR spectroscopy (**Figure 3**). From FT-IR analysis, functional groups and their interactions can be identified to determine encapsulate formation. From the results obtained, the overall visible spectrum between the extract and product encapsulation formulations have the same functional groups that are. The results of the FTIR spectral analysis of the ethanol extract of Jeruju leaves show that a peak at a wavelength of 3300-3500 cm^{-1} indicates the presence of the N-H amine/amide functional group, which usually appears at that wavelength. At a wavelength of 1610-1680 cm^{-1}, a peak indicates the presence of the C=C alkene functional group, which usually appears at that wavelength. Then, a peak appeared at a wavelength of 1050-1300 cm^{-1}, which might indicate the presence of the C-O Alcohol/ether/ carboxylic acid/ester functional group, which usually appears at that wavelength. At a wavelength of 675-995 cm^{-1}, a peak possibly indicates the presence of the C-H Alkene functional group, which usually appears at wavelengths 675-995 cm^{-1}.

The FTIR spectrum shows a wide absorption band at wave numbers 3200-3600 cm^{-1} which indicates a loosening of the O-H (hydroxyl) and wave numbers at 2850-2970 and 1340-14700 cm^{-1} indicate stretching the C-H bond. The encapsulation formulation and the coating material have almost the same spectrum, the peak appearing at a wavelength of 675-995 cm^{-1}, which indicates the presence of the C-H Alkene functional group, which usually appears at a wavelength of 675-995 cm^{-1}. The FT-IR spectra of Tween-80 showed a major band of C-H at 675-995 cm^{-1}, and the transmittance value decreased in the encapsulation results. Meanwhile, the major band in maltodextrin at 1050-1300 cm^{-1} C-O stretching, the same band appears in all encapsulates; this is the same as previous research (Wongverawattanakul et al., 2022). Theoretically, the occurrence of a cross-linking reaction between Ca^{2+} and maltodextrin affects the intensity of the asymmetry, and symmetry COO- stretching was observed at 1594 cm^{-1}, and a weak symmetric peak was presented at 1400–1500cm^{-1} Also a peak appearing at a wavelength of 1733 cm^{-1} indicates the presence of the C=O Aldehid/ketone/carboxylic acid/ester functional group, which usually appears at a wavelength of 1690-1760 cm^{-1}. The results of the comparison of the FT-IR spectra between the coating and the encapsulation results show that an encapsulation is formed, and it can be seen that all the spectra present in the encapsulation material appear on the encapsulation.

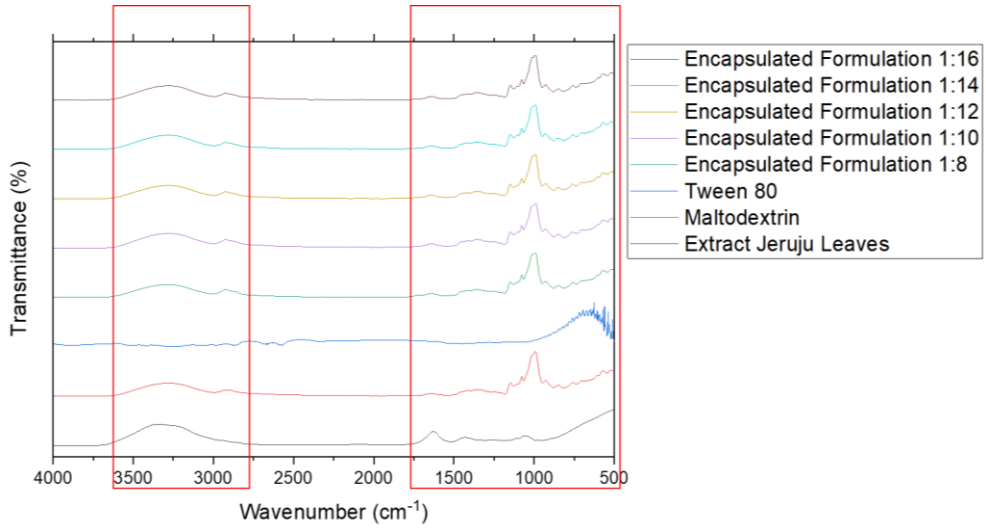

Figure 3. FTIR spectra of ethanol extract of jeruju leaves, encapsulation coating material and several encapsulation formulations

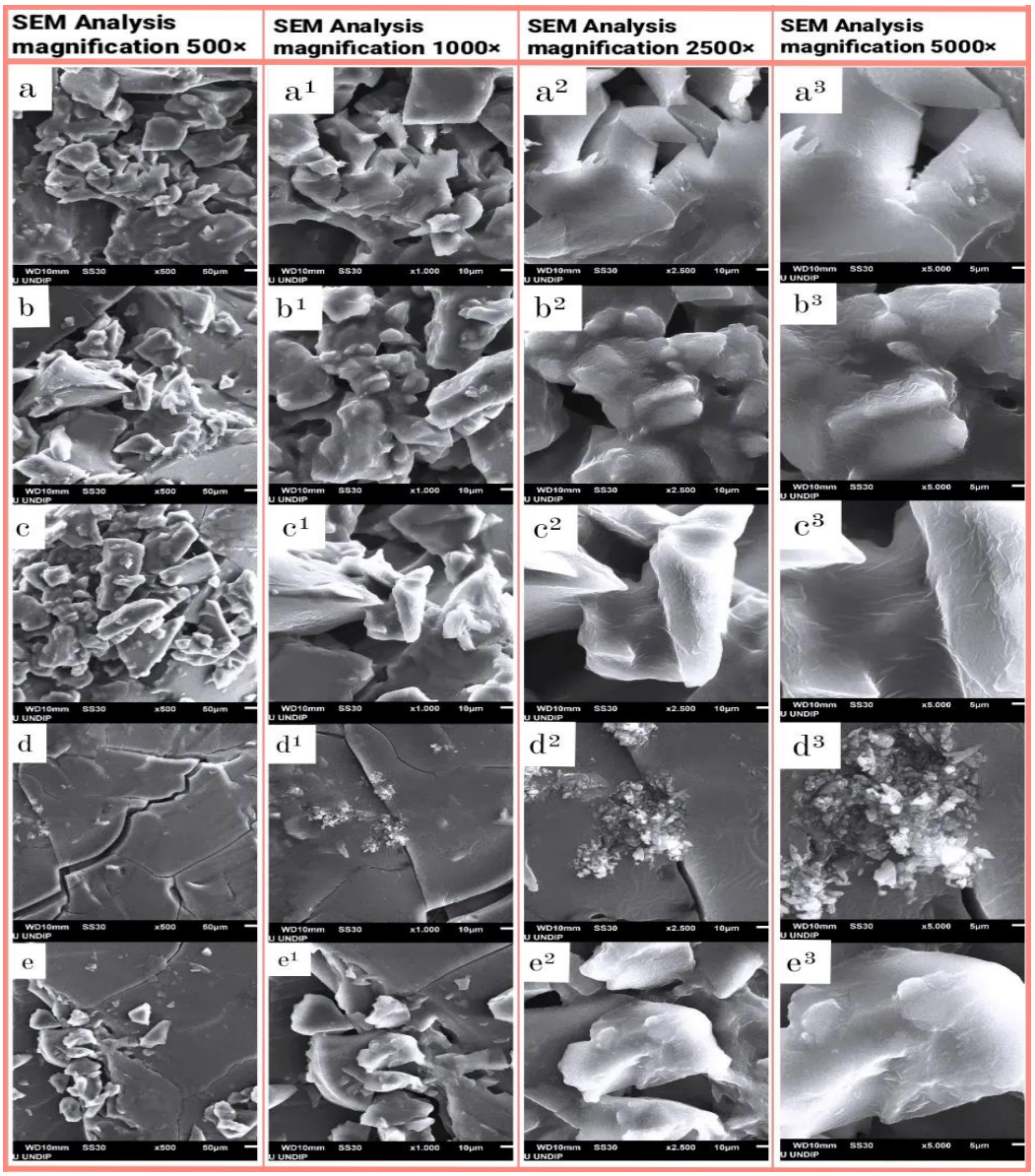

Figure 4. SEM analysis results on several encapsulation formulations

Scanning electron microscopy (SEM)

Morphological analysis was carried out using SEM at several magnifications, namely magnification 500×; 1000×; 2500×, and 5000× magnification. The **Figure 4** shows that the resulting particle structure is not uniform and tends to be in the form of flakes. This result is similar to the study of Alexandre et al (2019) which produced solids that resembled flakes and were not uniform. In addition, it is also seen that there is high agglomeration in all variables which can be caused by the high oil content on the surface (Bae & Lee, 2008). Agglomeration can also occur due to the low ratio of coating material to core material. When the amount of core material increases, a connecting bridge will form between the particles which in turn causes the particles to join (Alexandre et al., 2019). If the connecting bridge gets bigger, the distance between the particles becomes shorter and the particles will be more closely joined. In the final stage of this merger is the melting of the powder into a liquid.

Analysis using SEM to show the characteristics of the encapsulation formulation made. The morphology of the 1:8 encapsulation formulation as seen from SEM with 500× magnification has an irregular flake shape (**Figure 4**). The 1:10 encapsulation formulation on the SEM results at 1000× magnification looks not spherical. Meanwhile, in the 1:12 encapsulation formulation the magnification of 2500× encapsulation is formed non-spherically between the polymer and the active substance, this indicates that the 1:12 encapsulation formulation is encapsulated in the polymer. In the 1 : 14 encapsulation formulation at 2500× magnification, it was seen that there was a buildup between the encapsulations, where irregular spheres were formed, this was due to the use of too large a polymer so that in this formula accumulation occurred between the polymers and this could result in difficulty for the extract to be dispersed into the polymer solution so that it was not encapsulated. As well as in the 1:16 encapsulation formulation with a magnification of 1000× it can be seen that the encapsulation forms flakes, and the length is uniform but has not fully adhered because the polymer does not entirely encapsulate this formula with a ratio of 1:16.

Anti-inflammatory Activities

Statistic test one-way ANOVA showed a significant difference between the groups of mice tested ($p<0.05$). Because of the test Post Hoc LSD can be done to see which group has a significant difference. The tabulation table of test results post Hoc LSD is presented in **Table 2.** Test Post Hoc LSD in **Table 2** shows that all doses had statistically significant differences in inhibition compared to the negative control. Meanwhile, it was also seen that there was no significant difference between the percent inhibition of EE of 300, 600, and 900 mg/KgBW, which means that the three doses had almost the same percentage of inflammation inhibition. Compared with the positive control, all doses given significantly differed in the percentage inhibition value. This shows that the dose of the extract has anti-inflammatory potential. The inhibitory activity of the ethanol extract at a dose of 900 mg/KgBW reached 58.67%, still lower when compared to the control Na-Diclofenac which reached 82.33% (Setiawan et al, 2022).

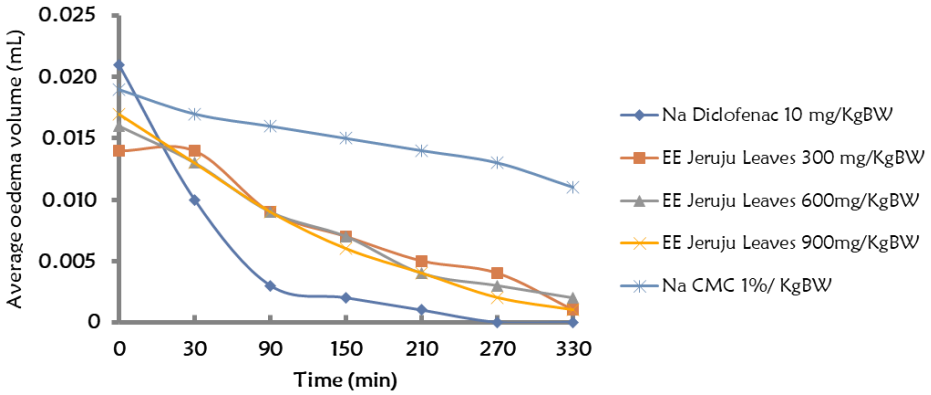

Figure 5. Graph of average oedema vs time (EE: Ethanol Extract of Jeruju Leaves)

Table 2. LSD Post Hoc test results on extract anti-inflammation tests

Substance And Dosage (mg/KgBW)	% Inhibition ± SEM
Na Diclofenac 10	$82.33^{a} \pm 0.05$
EE Jeruju Leaves 300	$52.18^{b} \pm 0.01$
EE Jeruju Leaves 600	$55.68^{b} \pm 0.01$
EE Jeruju Leaves 900	$58.95^{b} \pm 0.04$
Na CMC 1%	$0.00^{c} \pm 0.12$

Note: ^{a,b,c,d,e}Superscripts with different lowercase letters on the same line showed significant differences (Sig < 0.05)

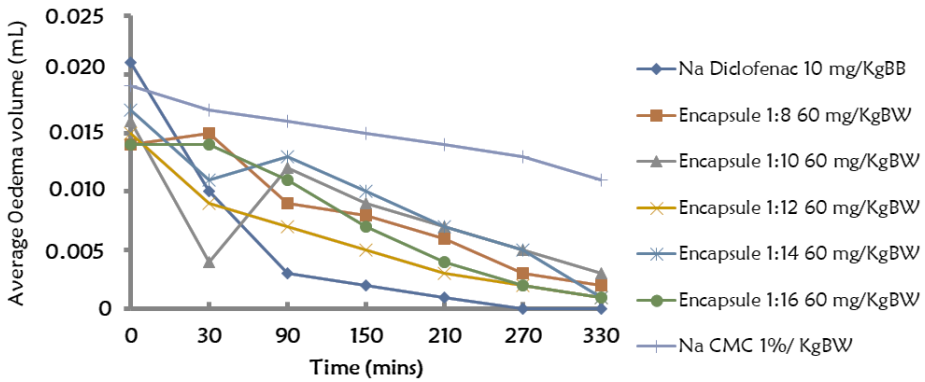

Figure 6. Graph of average oedema vs time

Figure 6 shows that the encapsulation extract ethanol and jeruju with several formulation ratios at a dose of 60 mg/KgBW can significantly reduce swelling on the legs of mice in the 60th minute after induction. However, there was an increase in oedema volume at the 60th minute in encapsulated formulations with a ratio of 1:8 and 1:16. In the 120th minute, an increase in the volume of oedema was also seen in the encapsulated formulations with a ratio of 1:10 and 1:14. Based on the data in Figure 13, the average percent inhibition was calculated at a dose of 60 mg/KgBW given. The average percentage of inhibition for encapsulated ethanol extract of Jeruju leaves in several formulation ratios such as 1:8 was 48.69%, at 1:10 was 50.22%, and at 1:12 respectively was 67.69%. Furthermore, the average percent inhibition of encapsulation in the formulation ratio 1:14 was 44.10%, and 1:16 was 53.93%.

Furthermore, an anti-inflammatory test was carried out with some encapsulation ratio at 90 mg/KgBW. **Figure 7** shows a significant decrease in swelling from several ratios of encapsulated formulations at 120 minutes. The average inhibition was obtained above 50% inhibition of inflammation for encapsulated ethanol extract of Jeruju leaves at a dose of 90 mg/KgBW from a ratio of 1:8; 1:10; 1:12, and 1:16 respectively, namely 50.87%, 57.64%, 69.43%, and 53.93% **(Table 3).** Meanwhile, the average inhibition percentage of encapsulation at a dose of 90 mg/KgBW below 50% was obtained at a formulation ratio 1:14, namely 47.38%. From a dose of 90 mg/KgBW, it can be seen that there is an increase in percent inhibition in the 1:12 encapsulation formulation was relatively high compared to the 1:10 encapsulation formulation, which was 69.43% **(Table 3).**

In the graphical results of **Figure 8**, the antiinflammatory test was carried out with several ratios of encapsulates at a dose of 120 mg/KgBW. Several ratios of encapsulated formulations at 120 minutes showed a significant reduction in swelling. The average inhibition was obtained above 50% inhibition of inflammation for encapsulated ethanol extract of Jeruju leaves at a dose of 120 mg/KgBW from a ratio of 1:10, 1:12, and 1:16, respectively 59.83%, 75.55%, and 62.01%. Meanwhile, the average percent inhibition of encapsulation at 1:8 and 1:14 obtained the same average percent inhibition, namely 53.28%.

The results obtained from the percentage of oedema inhibition of several ratios of encapsulated formulations with three doses of 60, 90, and 120 mg/KgBW were then analyzed by the one-way ANOVA to see the differences from each group. Due to conditions, one-way ANOVA is not fulfilled. It is continued with the LSD (Smallest significant difference) test with the method post hoc LSD to see significant differences between treatment groups ($\rho \leq 0.05$). Because of the test Post Hoc LSD can be done to see which group has a significant difference. The tabulation table of test results Post Hoc LSD is presented in **Table 3**.

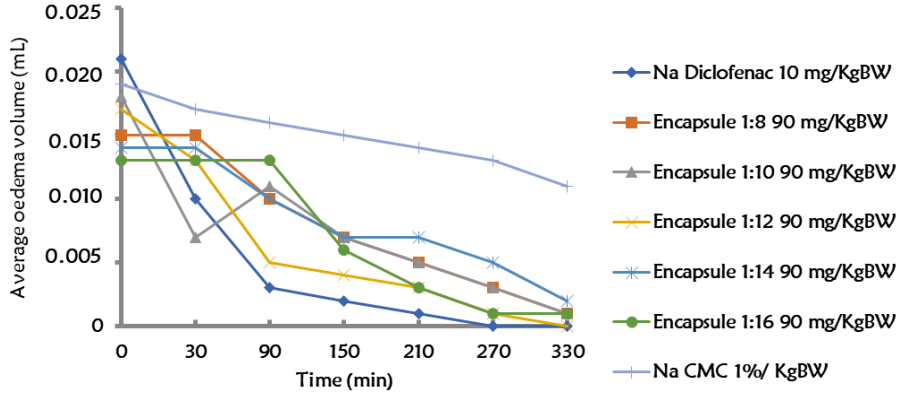

Figure 7. Graph of average oedema vs time

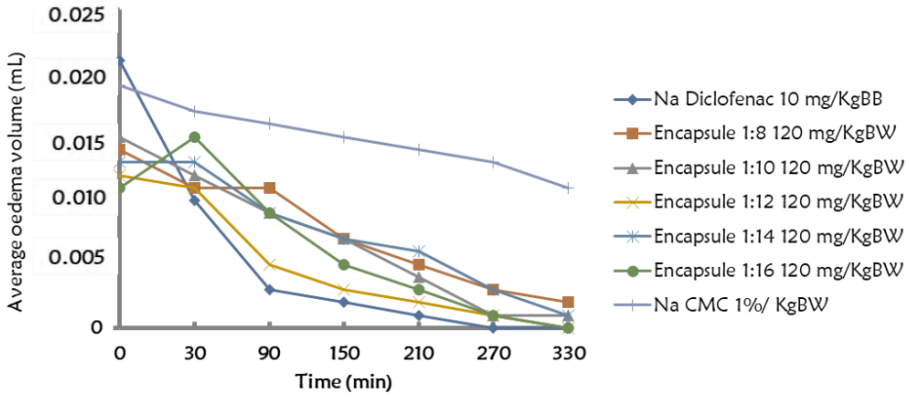

Figure 8. Graph of average oedema vs time

Table 3. Test Results *Post Hoc LSD* on Encapsule Anti-Inflammatory Testing

Substances	Dosages (mg/KgBW)	% Inhibition ± SEM
Na Diclofenac	10	$82.33^{a} \pm 0.05$
Encapsule 1:12	120	$75.33^{a,b} \pm 0.03$
Encapsule 1:12	90	$69.33^{a,b,c} \pm 0.11$
Encapsule 1:12	60	$67.67^{a,b,c,d} \pm 0.05$
Encapsule 1:16	120	$62.00^{b,c,d,e} \pm 0.01$
Encapsule 1:10	90	$61.00^{b,c,d,e} \pm 0.06$
Encapsule 1:10	120	$60.00^{b,c,d,e} \pm 0.11$
Encapsule 1:16	90	$56.67^{b,c,d,e} \pm 0.03$
Encapsule 1:16	60	$54.00^{c,d,e} \pm 0.02$
Encapsule 1:14	120	$53.33^{c,d,e} \pm 0.03$
Encapsule 1:8	120	$53.00^{c,d,e} \pm 0.07$
Encapsule 1:8	90	$51.00^{c,d,e} \pm 0.03$
Encapsule 1:10	60	$50.33^{c,d,e} \pm 0.01$
Encapsule 1:8	60	$48.67^{d,e} \pm 0.03$
Encapsule 1:14	90	$47.33^{e} \pm 0.07$
Encapsule 1:14	90	$44.00^{e} \pm 0.05$
Na CMC 1%	10	$0.00^{f} \pm 0.12$

Note: ^{a,b,c,d,e}Superscripts with different lowercase letters on the same line showed significant differences (*Sig* < 0.05

Test results data post hoc LSD In **Table 3**, it can be seen that all encapsulation formulation ratios statistically have a significant difference in percent inhibition compared to the negative control. This shows that all ratios of encapsulated formulations are active as anti-inflammatory. It was also seen that there was no significant difference between the inhibition of encapsulation 1:8 60 mg/KgBW, encapsulation 1:14 60 mg/KgBW, and Encasulation 1:14 90 mg/KgBW. Likewise, the percentage inhibition of encapsulation was 1:8 90 mg/KgBW, 1:8 120 mg/KgBW, 1:10 60 mg/KgBW, 1:14 120 mg/KgBW with encapsulation 1:16 90 mg/KgBW. And so it is in the encapsulation 1:10 90 mg/KgBW, 1:10 120 mg/KgBW, 1:16 90 mg/KgBW with encapsulation 1:16 120 mg/KgBW, as well as Encapsule 1:12 90 mg/KgBW with Encapsulation 1:12 60 mg/KgBW. This indicates that formulation encapsulation has the same inflammatory inhibition power between several ratios. This similarity in inhibition occurs because there is a possibility that the same chemical compounds are present in several ratios of encapsulated compound formulations (Karim et al., 2019).

This study used multilevel doses to know the right encapsulated formulation dose that could show optimal anti-inflammatory effects. The effectiveness of encapsulation in reducing oedema can be seen from the average percentage of oedema. In **Table 3**, encapsulation 1:12 120 mg/KgBW has a significant percentage of inhibition with Diclofenac Na 10 mg/KgBW. This is because the higher the concentration, the higher the percentage of inhibition, and the ability to inhibit inflammation will also increase, indicating a high percentage of inhibition at that optimum concentration (Kadji *et al*., 2015). This can be seen in the anti-inflammatory test of extracts that have not been encapsulated. However, with increasing dose or concentration, the anti-inflammatory activity will increase. However, in encapsulated formulations with a ratio of 1:14 and 1:16, there was a decrease in anti-inflammatory activity. This is because there are several types of drugs

in higher doses that cause the release of histamine directly from mast cells, causing blood vessels to become more permeable to plasma fluid and causing an inflammatory process (Baldo & Pham, 2012).

CONCLUSIONS

There are differences in the physical and chemical characteristics of the extract before it is encapsulated and the extract encapsulated formulation. Where the encapsulation formulation has a percent value of product yield and encapsulation solubility in water is better than the ethanol extract of *A. ilicifolius* leaves. The best antiinflammatory activity in the 1:12 formulation with a dose of 120 mg/KgBW obtained a high inhibition percentage of 75.55%, indicating anti-inflammatory activity whose inhibition percentage value was close to the positive control of Diclofenac Na, namely 80%.

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