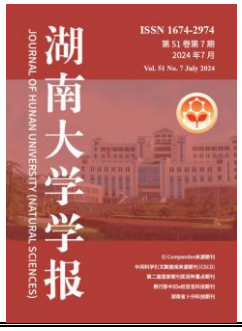

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Encapsulation of Ethanol Extract *Sonneratia Alba* Leaves with Inulin Matrix Coating as an Antioxidant Supplement

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Abstract: Perepat (*Sonneratia alba*) is a mangrove species widely reported to have potential as a medicinal plant. Encapsulation in coating technology has been developed to prevent bioactive damage and to improve bioavailability. This study aimed to analyze the characteristics of ethanol extracts of *Sonneratia alba* (*S. alba*) leaves encapsulated using an inulin coating and to determine the encapsulation ability of ethanol extracts from perepat leaves. The highest total phenol and flavonoid extract levels were obtained in PKL at 171,880 mg GAE/g and 25,473 mg QE/g, respectively, in line with its antioxidant activity (IC_{50} 3,544 µg/mL). The highest total phenol and flavonoid levels were obtained in PKL encapsulating 38.963 mg GAE/g and 5.583 mg QE/g, respectively, with an IC_{50} of 91.571 µg/mL. IR results of encapsulated ethanol extracts of *S. alba* leaves from four different regions showed the presence of C-H alkane functional groups, C-H alkene functional groups, C-H aromatic ring functional groups, O-H functional groups, hydrogen/phenol bond alcohols, C=C alkene functional groups, and C-O functional groups of alcohol/ether/carboxylic acids/esters. The IR spectrum confirms the cross-linking reaction and the formation of the Encapsule. SEM produces particles of various sizes, irregular surface structures, and deep hollows in walls. The results of in vitro bioavailability testing of ethanol extract encapsulation from perepat leaves with inulin coating of PKL showed an increase in the percentage of drug release in the pH buffer 1.2 from the previous minute to minute 120, which increased to 41.41%. In the pH buffer 7.4 from the last minute to minute 240, there was an increase in drug release, reaching 98.54%. This finding shows that encapsulation of the extract has good potential as a capsule supplement.

Keywords: antioxidant, bioavailability, encapsule, *Sonneratia alba*.

将海桑叶乙醇提取物与菊粉基质涂层一起封装，作为抗氧化补充剂

摘要：桑树(白海桑)是一种红树种，据广泛报道具有药用潜力。包衣技术已得到发展，以防止生物活性损伤并提高生物利用度。本研究旨在分析使用菊粉包衣包衣的桑树(白海桑)叶乙醇提取物的特性，并确定桑树叶乙醇提取物的包衣能力。PKL中的总酚和黄酮类提取物含量最高，分别为 171,880毫克GAE/克和 25,473毫克量化效应/克，与其抗氧化活性(IC50 3,544微克/毫升)相符。PKL包封的总酚和黄酮含量最高，分别为38.963毫克GAE/克和5.583毫克量化效应/克，IC50为91.571微克/毫升。来自四个不同地区的白花蛇舌草叶包封乙醇提

取物的IR结果显示存在C-H烷烃官能团、C-H烯烃官能团、C-H芳环官能团、O-H官能团、氢/酚键醇、C=C烯烃官能团和醇/醚/羧酸/酯的C-O官能团。红外光谱证实了交联反应和包封的形成。SEM产生各种尺寸的颗粒、不规则的表面结构和壁中的深凹陷。用PKL菊粉涂层包覆的佩雷帕特叶子乙醇提取物的体外生物利用度测试结果显示，在pH缓冲液1.2中，从前一分钟到第120分钟，药物释放百分比增加，增至41.41%。在pH缓冲液7.4中，从最后一分钟到第240分钟，药物释放增加，达到98.54%。这一发现表明，提取物的包覆具有作为胶囊补充剂的良好潜力。

关键词：抗氧化剂，生物利用度，胶囊，白海桑。

1. Introduction

Mangrove plants are vegetation plants found in several regions of Indonesia [1], especially Sumatra. Jambi Province and the Riau Islands have a wealth of local plants, especially mangroves [2]. One area that has many mangrove plants in Jambi Province is the East Tanjung Jabung region, and in the Riau Islands region, there are three areas, namely Pengudang, Sebong Lagoi, and Dompak. Perepat (*Sonneratia alba*) is a plant that is generally found in sandy loam areas around the coast, along river banks, or swamps that are still influenced by tides [1]. Based on previous research Muhaimin et al. [3] reported that *S. alba* leaves contain alkaloids, phenolics, triterpenoids, flavonoids, tannins, saponins, steroids, and glycosides. *S. alba* contains flavonoids and phenolics, which are bioactive compounds that can be used as antioxidants [3, 4].

The stability of bioactive compounds is strongly influenced by external factors such as air, temperature, and light [5]. This high-temperature effect damages the structure of the chemical compounds. Organic compounds, such as flavonoids and phenolic components, are sensitive to heat and are quickly oxidized [6]. The higher the temperature and duration of the heat treatment, the lower the phenol content and antioxidant capacity [5, 7]. Encapsulation is a technique of coating active ingredients in the form of solids, liquids, and gases to protect the core material in the form of bioactive compounds from various environmental influences. Substances that are not resistant to environmental influences, such as proteins, enzymes, and other compounds, can be maintained using encapsulation techniques [8].

Encapsulation by freeze-drying is a low-temperature drying method. The basic principle of freeze-drying is the process of removing the water content in a material or product that has been frozen without going through the liquid phase [9]. Several encapsulation methods based on particle size include freeze-drying, spray-drying, cooling, fluid-bed coating, coacervation, and liposome entrapment [10]. Freeze drying has advantages, especially for heat-sensitive products; it can maintain product stability (avoiding changes in aroma, color, and other elements). The results of freeze-drying also have physiological, organoleptic, and physical shape properties that are almost the same as before drying [11]. In the encapsulation process, a coating material is used, and one of the good coating materials is inulin because it has food fiber in the form of oligosaccharides (monomers in the form of fructose) derived from tubers, has a high fiber content, and is widely used because of its high solubility and emulsifying ability [12].

Developing bioactive antioxidants into supplement products is essential to know in advance the amount of active substances in the ethanol extract encapsulant from *S. alba* leaves, which functions as an antioxidant that can be digested and absorbed by the body (bioavailability). The encapsulated bioavailability test of the ethanol extract of *S. alba* leaves was carried out in vitro using a pH buffer. This study aimed to analyze the chemical and physical properties of the ethanol extract of *S. alba* leaves, encapsulation with an inulin coating as an antioxidant supplement, and encapsulation of the ethanol extract from *S. alba* leaves.

2. Material and Methods

2.1. Material and Instrumentation

The materials used in this study were *S. alba* leaves obtained from four regions: Tanjung Jabung Timur Jambi Province, Pengudang, Sebong Lagoi, and Dompak Riau Islands Province, ethanol, Folin-Ciocalteu reagent, Na_2CO_3 7%, methanol (p.a), gallic acid, $AlCl_3$, sodium acetate, quarttin, ascorbic acid, DPPH ((1,1-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Singapore)), Aquadest, Inulin, tween-80, pH buffer 1.2, and pH buffer 7.4. A UV-Vis spectrophotometric spectrophotometer (Thermo-Fisher Orion Scientific AQ8100, Waltham, MA, USA) with a wavelength of 200-800 nm and FTIR spectrophotometer (Thermo Fisher Scientific) at wavenumbers 4000-400 cm-1 and SEM (Hitachi SU-

3500) magnifications of 500, 1000, 2500, and 5000 × (Times) were used.

2.2. Methods

2.2.1. Sample Preparation

S. alba leaves were shorted, washed, and aerated to a moisture content of 10%. *S. alba* leaves were mashed and filtered through a 60-mesh sieve to obtain a Simplisia powder. Simplisia was extracted with ethanol (70% v/v) (ratio 1:10) by maceration for 2 × 24 h. The filtrate was filtered through Whatman No.1 and evaporated using a rotary vacuum evaporator (Buchi) at 50°C to obtain the crude extract [13].

2.2.2. Extract Yield

The yield is calculated following previous studies [8] with the following formula 1.

%Yield Extract = WE/WS x 100% (1)

Notes:

WE - weight of extract (g)

WS - weight of simplicia (g)

2.2.3. Encapsulation

Encapsulation was initiated by preparing emulsions and was performed with inulin coatings. The inulin solution was prepared by adding H2O at ratio 1:1 (ddH2O: inulin). The emulsion formulation had a ratio of 8:1 (inulin coating: extract). The samples in this study were divided into four groups (Table 1).

Table 1 Sample codes (Developed by the authors)

Sample Codes	Information
PKL	Perepat Kampung Laut, Tanjung Jabung Regence, Jambi Province
PPG	Perepat Pengudang, Batam, Riau Islands
PLG	Perepat Lagoi, Batam, Riau Islands
PDP	Perepat Dompak, Batam, Riau Islands

The mixture was homogenized using a magnetic stirrer at room temperature at 700 rpm. Tween 80 was added in 20 drops (the homogeneous process was carried out for 1 h). It was then dried using a freeze dryer for 8 h. After drying, it was mashed using a mortar and pestle to produce the encapsulant powder [12]. Encapsules were characterized, including percentage yield, water content, solubility in water, total phenols and flavonoids, antioxidant activity, encapsulation efficiency, analysis using FTIR, and morphology using SEM.

2.2.4. Percent Encapsulant Yield

The yield is calculated following previous studies (8) with the following formula 2.

% Yield = WE (mg)/WRM X 100% (2)

Notes: WE - weight of encapsule; WRM - weight of raw materials

2.2.5. Encapsules Moisture

The encapsulated water content of the ethanol extract from the perepat leaves was measured using the thermogravimetric method. The water contained in food is evaporated by heating, during which the material is weighed until the weight becomes constant, so that the water content in the food has been lost or evaporated. Empty weighing bottles coded for each sample were heated at 105 °C for 4 h, with the bottle cap half open. After 4 h, an empty weighing bottle was taken from the oven, placed in a desiccator for 15 min to remove any remaining moisture, and then weighed as weight A. The encapsulated samples of crude extract were each weighed as much as 1 g and placed in a weighing bottle that had previously been weighed and is known as weight B. The weighing bottle, along with the encapsulated sample of crude extract of perepat leaves, is then put into an oven that has been set to 105°C for 3-4 hr. It was then placed in a desiccator for 15 min and weighed as C. The percentage water content in each sample was calculated using the following formula 3.

%Ka = (B-C)/(B-A) (3)

Notes:

Ka - percent moisture content (%)

A - weight of empty weighing bottle (g)

B = Weight of Weighing Bottle + Initial Sample (g)

C = Weight of Weighing Bottle + Weight of Dry Sample (g)

2.2.6. Encapsules Solubility

The solubility was determined according to a previous study with slight modifications [14]. One gram of the encapsule was placed in 10 mL of water at 30^0C and stirred using a magnetic stirrer. The solution was then filtered using Whatman filter paper, which has a known fixed weight. The filter paper and unfiltered parts of the sample were placed in an oven for 1 hour at 1050C, then cooled in a desiccator for 15 min, and then weighed. The weight of the unfiltered sample was obtained from the difference between the weights of the final and initial filter papers. Solubility was calculated using formula 4.

$$\text{Solubility (\%)} = \left(1 - \left(\frac{(c-b)}{a\ x \frac{100\ x\ Ka}{100}}\right)\right) \times 100\% \quad (4)$$

Notes:

a - sample weight used (g)

b - filter paper weight (g)

c - filter and residue paper weights (g)

Ka - moisture content of the sample

2.2.7. Total Phenol Content (TPC)

Samples (1 mL) were added with 0.5 mL of Folin-Ciocelteu reagent, shaken, and incubated for 4-8 minutes. Furthermore, 4 mL of 7% Na_2CO_3 was added, and the mixture was shaken gently. Aquades (10 mL) were added and the solution was incubated at room temperature for 2 h. Absorbance was measured using a

UV-Vis spectrophotometer at a maximum wavelength of 744.8 nm. Total phenol was calculated using the linear regression equation of the previously measured gallic acid calibration curve, which was prepared at concentrations of 10, 20, 30, 40, and 50 ppm, prepared by dilution in methanol p.a. TPC was calculated using the following formula 5.

TPC = (C.V.df/s) (5)

Notes:
TPC - total phenolic content (mgGAE/g)
C - sample concentration (mg/mL)
V - volume of extract used (ml)
df - dilution factor
s - sample weight (g)

2.2.8. Total Flavonoid Content (TFC)

The total flavonoid content of the extract and encapsulants was measured as previously described, with slight modifications. Briefly, 80 µL of the sample was mixed with 80 µL 2% aluminum chloride and 120 µL sodium acetate solution (50 g/L) and incubated for 30 min at room temperature. The absorbance was measured at 431 nm using a calibration curve of quercetin in an alcohol solution with concentrations ranging from 0 to 50 µg/mL. The results were obtained by subtracting it from the blank and expressed as milligrams of quercetin equivalents (QE) per gram of coffee (mg QE/g) ± standard deviation (SD). The standard solution used was quercetin (p. a.) dissolved in methanol to produce a series of concentrations of 0, 20, 30, 40, and 50 ppm, and a linear regression calibration curve was obtained [14]. The TFC was calculated using Formula 6.

TFC = (C x v/m) x df (6)

Notes:
TFC- total flavonoid content (mgQEg)
C - quercetin concentration (mg/mL)
Df - dilution factor
m - extract weight (g)
V - volume of extract (L)

2.2.9. Antioxidant Activity

The antioxidant activity was analyzed using the DPPH method (1.1-diphenyl-2-picrylhydrazyl). Antioxidants were carried out in triplicate using ethanol as a blank with four concentration variations of the extract and encapsulation. The samples were prepared at concentrations of 10, 30, and 50 ppm. Ascorbic acid was used as the positive control. The antioxidant activity was determined by 0.2 ml added to 3.8 mL of 50 µM DPPH solution. The mixture was homogenized and incubated for 30 min in a dark room. The absorbance of the solution mixture was measured using UV-visible spectrophotometry at a wavelength of 517 nm [13]. The percent inhibition value indicated DPPH dampening activity, and the IC_{50} value indicated the concentration that resulted in 50% inhibition. The % inhibition value was determined using Equation 7.

% Inhibition = (CA – SA)/ CA x 10% (7)

Notes:
CA - control absorbance
SA - sample absorbance

2.2.10. Encapsulation Efficiency

The encapsulation efficiency is determined using Formula 8.

% Encapculation Efficiency = $\frac{A}{B}$ x 100% (8)

Notes:
A - total encapsulated phenol content
B - total phenol content before encapsulation

2.2.11. IR Spectrum and Morphology Analysis

The infrared spectrum was analyzed against inulin coating, encapsulation, and Tween-80. The results of the analysis were recorded using a 640-IR. FTIR Variant spectrophotometer at wavenumbers of 400-4000 cm^{-1}. The morphology was analyzed using Scanning Electron Microscopy (SEM-EDX JEOL JSM-6510LA) at magnifications of 500, 1000, 2500, and 5000 ×.

2.2.12. Encapsulant Bioavailability

The encapsulation bioavailability test was performed in vitro. The encapsulation standard curve was determined by measuring the absorption of the encapsulation solution in a phosphate buffer medium (pH 1.2) with concentrations of 10, 20, 30, 40, and 50 ppm, and in a phosphate buffer solution medium of pH 7.4, with concentrations of 10, 20, 30, 40, and 50 ppm. An in vitro dissolution test was conducted by inserting 500 mg of encapsulated perepat leaf ethanol extract microcapsules into a container containing 10 mL of phosphate buffer pH 1.2 and 7.4 at room temperature. Samples were analyzed at 30, 60, and 120 min intervals, and short images of the phosphate buffer pH 7.4 were taken at intervals of 30, 60, 120, and 240 min. The absorbance of the filtrate was measured at the maximum wavelength using a UV-Vis spectrophotometer.

2.3. Statistical Analysis

Data were analyzed using Excel and GraphPad Prism for Windows version 5 (Graph Pad Software Inc., San Diego, CA, USA) using one-way ANOVA. Statistical significance was set at p value < 0.05. All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

3. Results and Discussion

The extract yield was obtained from the weight of the extract and the weight of simplisia. The yield of the extract is related to its bioactive content. The higher the extract yield, the higher the yield [15]. The extract yield was calculated as percentage (%), as shown in Fig. 1.

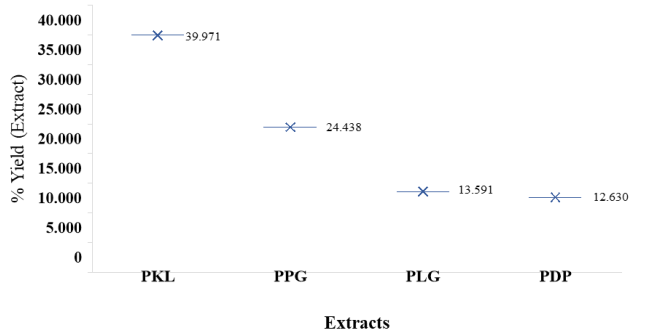


Fig. 1 Extract yield (%) (Developed by the authors)

Based on the yield results, it can be assumed that the bioactive components contained in the PKL area were higher than those in the others (Fig. 1). The secondary metabolite content is influenced by environmental factors such as light, available nutrients, medium composition, morphological differences, plant tissues used, and biosynthetic activities [15]. In addition, the growth of biota is influenced by both external and internal factors. External factors, such as habitat, season, water temperature, and type of food, were available. Internal factors, such as age, size, and other biological factors. The chemical compound content in a plant is greatly influenced by its geographical location, temperature, climate, and soil fertility. The composition of chemical components varies from region to region within the same plant [15].

3.1. Encapsule Characteristics

The encapsulated yield was obtained by drying the encapsulated weight of the ethanol extract from the obtained perepat leaves and then comparing it with the weight of the emulsion before drying. The results are obtained in the form of a percentage (%), as shown in Fig. 2. The encapsulated PDP, PLG, PPG, and PKL obtained with inulin coating were 52.04%, 55,21%, 53.56%, and 59.54%, respectively. Some factors that cause high and low yields are age, water content, and inulin content of the encapsulants. The higher the inulin content in the encapsulant, the more dissolved the precipitate, and the higher the yield. The greater the concentration of the coating, the greater the yield of the product obtained [21]. Encapsulation efficiency is related to the properties of the coating material, such as its film-forming ability and stable emulsion-forming ability. The encapsulation material and inlet temperature most often affect the encapsulation efficiency [16, 17].

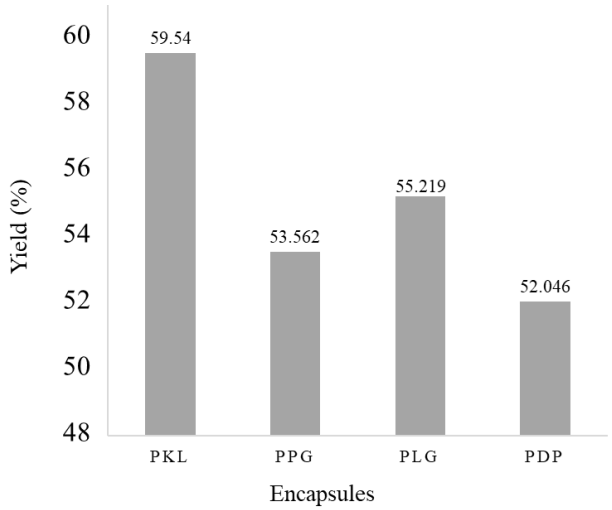


Fig. 2 Percent encapsulant yield (Developed by the authors)

3.2. Encapsulant Water Content

The moisture content was calculated as the difference between the initial and final weights, divided by the initial weight, and multiplied by 100%. The results are obtained in the form of percent (%), as shown in Fig. 3.

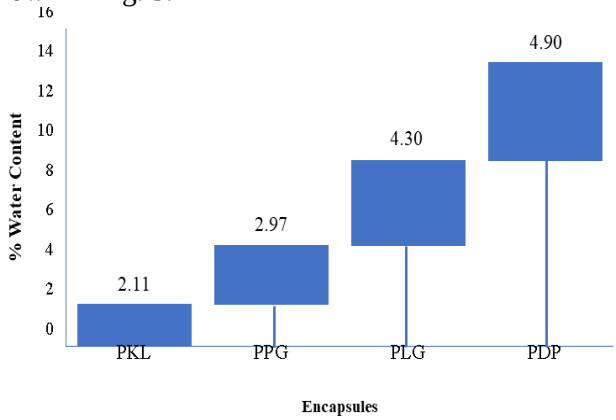


Fig. 3 Encapsulant water content (Developed by the authors)

The water content of ethanol extract encapsulation from perepat leaves with inulin coating ranged from 2.11% to 4.90%. Overall, the moisture content of a material is influenced by the temperature and duration of grinding. The smaller the water content of a material, the smaller the weight of water contained in the material, which is the main component. If water is removed, the material becomes denser and lighter, thus affecting the final product. Previous research reported that the study's results showed that the higher the concentration of maltodextrin and tween 80, the lower the water content [18]. The interaction between maltodextrin and tween-80 can cause a decrease the moisture content of the material.

Maltodextrin is composed of free hydroxyl groups, and tween-80 has hydrophilic properties, allowing it to easily bind water to the material [19]. During the drying process, water bound together with maltodextrin and Tween 80 was more volatile, reducing the moisture content of the resulting product. Maltodextrin has a simple molecular structure; therefore, bound water and

free water can be easily removed during drying [20]. Water content can damage food because it can cause chemical changes. Higher moisture content also triggers the growth of bacteria and fungi, which can cause damage to the dry product. The water content of a material is influenced by several factors, including the encapsulant material, drying process duration, drying process, and drying process.

3.3. Solubility of Encapsules

Solubility indicates the ability of a powder to dissolve in water. The results are shown in Fig. 4.

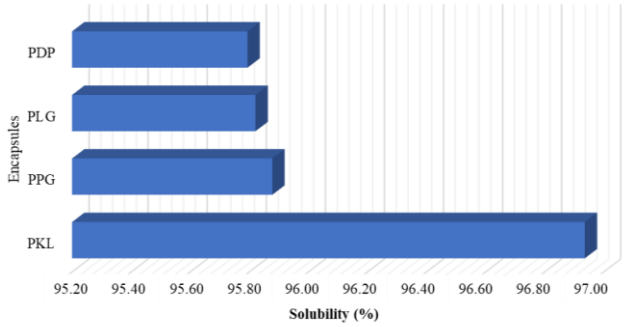


Fig. 4 Solubility of encapsules (Developed by the authors)

The results showed that the ethanol extract of *S. alba* leaves that passed the encapsulation process was readily soluble in water. The solubility of products encapsulated in water ranged from 95.79% to 96.93%. The solubility of all powder-form products is above 95% because inulin is a water-soluble polysaccharide that forms an evenly dispersed solution. The solubility of inulin ranges from 96.51% to-99.02%, indicating that inulin has high solubility in water [18]. The high and low percentage of solubility of the encapsulated products is thought to be due to the influence of the coating material in the form of polysaccharide compounds whose wrinkle properties are different from those of water. The coating properties affect the encapsulation activity in delivering the active substances contained therein [12].

3.4. Total Phenols and Encapsulated Flavonoids

The total phenolic content of the extract PKL is 171.880 mg GAE/g, PPG was 41.698 mg GAE/g, PLG was 38.286 mg GAE/g, and PDP was 37.036 mg GAE/g. The results showed that differences in growing areas and environmental conditions affected the total phenol levels in PKL > PLG, PPG, and PDP (Table 2). This result is in accordance with previous research (21), which showed that bioactive compounds depend on the cultivar. The results of the same study were also reported by Liu et al. [22], who showed that the height of the location affects the phenol content of *Potentilla fruticosa* (L) leaves. In addition to the height of the place that can affect the growth of a plant, the factors that can affect the production of secondary metabolites are environmental conditions [23]. Altitude differences affect the microclimate of an area. Environmental conditions, such as air temperature, soil temperature, humidity, and soil type, are part of the height of the place [21].

Table 2 Total phenol and flavonoid content (Developed by the authors)

Types	Samples	TPC mgGAE/g	TFC mgQE/g
Extract	PKL	171.880[a]	25.473[c]
	PPG	41.698[b]	8.376[d]
	PLG	38 .286[b]	8.269[d]
	PDP	37.036[b]	4.613[d]
Encapsules	PKL	38.963[a]	5.583[c]
	PPG	30.463[b]	5.067[c]
	PLG	27.588[b]	4.465[c]
	PDP	24.629[b]	3.948[c]

Note: Superscripts with different lowercase letters on the same line indicate significant differences ($p < 0.05$).

The total flavonoid content of PKL, PLG, and PDP extracts was 25,473 mgQE/g each, 8,376 mgQE/g, 8,269 mgQE/g and 4,613 mgQE/g. The results showed that differences in growing areas and environmental conditions affected the total phenol levels of PKL more than other samples (Table 2). Under environmental conditions with high temperatures, there is an increase in free radicals in the form of reactive oxygen species (ROS) in reactive plants in plant tissues that trigger cell damage. Plants produce antioxidants as a form of adaptation to high environmental temperatures [21]. At higher temperatures, higher total flavonoids will produce a higher total flavonoid as an extra synergy of defense against environmental stress [24].

The total Encapsulant phenolics were obtained by re-performing the total phenolic test, but this time on encapsulants with a concentration of 1000 ppm with a maximum wavelength of 744.8 nm. The total levels of the encapsulated phenolic compounds PKL, PPG, PLG, and PDP were 38.9625 (mgGAE/g), 30.4625 (mgGAE/g), 27.5875 (GAE/g) and 24.6292 (GAE/g) respectively. These results showed that the content of phenol compounds in the encapsulant form was lower than that in the extract form.

Total encapsulant flavonoids were obtained by testing encapsulants from the PKL, PPG, PLG, and PDP areas at a concentration of 1000 ppm, with a maximum wavelength of 431 nm. The total flavonoid levels in PKL, PPG, PLG, and PDP were 5.583 (mgQE/g), 5.067 (mgQE/g), 4.465 (mgQE/g) and 3.948 (mgQE/g), respectively. From these results, it is known that the TPC of the extract is greater than that of the encapsulants. The total phenol yield, with the highest total flavonoid content, was observed in the extract form rather than in the encapsulated form. Flavonoids are one of the most abundant classes of phenolic compounds in plants. In this study, the samples used in the total phenol and total flavonoid tests were the same, resulting in a total value that was not significantly different. Phenol compounds and flavonoids function as antioxidants in the body. The

ratio of total flavonoids to encapsulants is lower than that of extracts; in addition to being affected by encapsulation of nanoparticles, it can also be affected by the surfactants used. Surfactants play a role in increasing the solubility of a substance by lowering the surface tension between the solute and medium based on the formation of an agrrate called a micelle.

3.5. Antioxidant Activity

Antioxidant activity was determined by measuring the absorbance value at a wavelength of 517 nm and then converted into IC_{50} values. The antioxidant activity of the samples was determined by the magnitude of DPPH inhibition by percent inhibition. Percent inhibition in free-radical immersion is the ability of an antioxidant to inhibit free radicals related to the concentration of the sample tested. The greater the percentage of sample inhibition, the smaller the IC_{50} value.

The test results showed that the percent IC_{50} ethanol extract of *S. alba* leaves from PKL, PPG, PLG, and PDP at concentrations of 10, 20, 30, 40, and 50 µg/mL had IC_{50} values of 3.544, 5.482, 46.659, and 68.281 µg/mL, respectively. Based on the IC_{50} value, the ethanol extract of PKL leaves, PPG, and PLG had antioxidant activity with a very strong category or <50 µg/mL, while PDP had antioxidant activity with a strong category (IC_{50} value of 50-100 µg/mL). The results showed that differences in the growing region and environmental conditions affected the antioxidant activity (Table 3). The environmental conditions at low and high altitudes can encourage the formation of secondary metabolites that are higher than those at medium altitudes. The presence of antioxidant compounds in plant organs is a result of plant metabolism. The altitude of the location has the greatest influence on plant metabolism related to the availability of sunlight, ambient temperature, and nutrients.

Table 3 Antioxidant activity (IC_{50}) extract (Developed by the authors)

Types	Samples	IC_{50} ± SD
Extract	PKL	3.544 ± 0.001^{a}
	PPG	5.482 ± 0.002^{b}
	PLG	$46{,}659 \pm 0.002^{c}$
	PDP	$68{,}281 \pm 0.005^{d}$
Encapsules	PKL	91.571 ± 0.01^{e}
	PPG	494.213 ± 0.02^{f}
	PLG	503.974 ± 0.01^{g}
	PDP	550.271 ± 0.01^{h}

Note: Superscripts with different lowercase letters on the same line indicate significant differences ($p < 0.05$).

The antioxidant activity of the capsules was determined using the DPPH method with concentration variations and was measured at a maximum wavelength of 517 nm. Antioxidant activity results are shown in Table 3. A linear regression equation was obtained by plotting the concentration (x-axis) against the percent inhibition (y-axis). The linear regression equation for each encapsulant was obtained using different methods. PKL (y = 0.0541x + 45.046; R2= 0.946), PPG (y = 0.0197 + 40.264; R2 = 0.9397), PLG (y = 0.0234 + 38.207; R2 = 0.8872), PDP (y=0.0221+37.839; R2=0.8309).

There was a decrease in the IC_{50} encapsulating antioxidant activity compared to the extract in all samples. As with the total yield of phenols and flavonoids, the best antioxidant activity was found in the extract form rather than in the encapsulated form. Phenol compounds and flavonoids function as antioxidants in the body. The ratio of total flavonoids to encapsulants is lower than that of extracts; in addition to being affected by encapsulation of nanoparticles, it can also be affected by the surfactants used. Surfactants are often used as additives owing to their ability to emulsify, suspend, and dissolve drugs. In this study, Tween 80 was used as the surfactant.

3.6. Encapsulation Efficiency

The encapsulation efficiency indicates the amount of extract that can be coated by the coating. The higher the EE, the greater the coating of the extracts. The EE results of encapsulation efficiency are shown in Fig. 5.

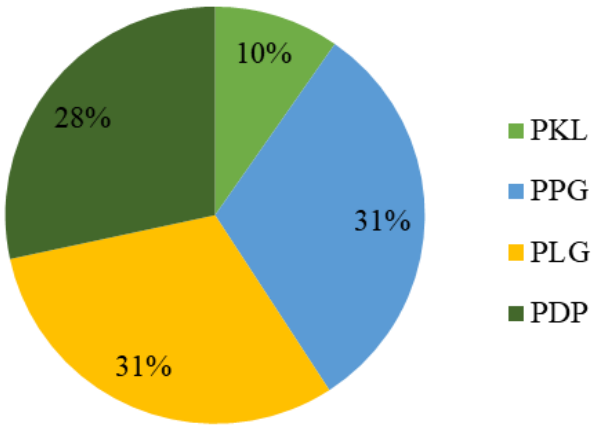


Fig. 5 Encapsulation efficiency pie graph (Developed by the authors)

The highest encapsulation efficiency of the PPG was 73.05%. The results of this efficiency indicated that there were still extracts that were not coated during the encapsulation process. The efficiency value was below 90%, caused by several factors, such as the nature of the coating material (viscosity and solubility), the ratio of the core material to the coating, and the inlet air temperature [25]. Inulin has high viscosity; it resembles a gel and forms gel particles after stirring. Fructans stirred at a high speed form a structure similar to a white cream. The strength of the gel formed from inulin depends on its concentration, stirring speed, and temperature. The factor that causes a high encapsulation efficiency is the relationship between the coating material and the core material. The greater the number of coatings, the greater is their ability to withstand the discharge rate.

3.7. IR Spectroscopy and Morphology

Characterization using an FTIR spectrophotometer was carried out to determine the functional groups present in the encapsulation of ethanol extract from *S. alba* leaves and to determine the presence of functional groups in encapsulated coating materials such as Tween-80, inulin, and samples encapsulated with encapsulated coating materials. From the results obtained as a whole, the spectrum seen between each encapsulant sample can be said to have functional groups that are not significantly different only in their wavenumbers, as shown in Fig. 6.

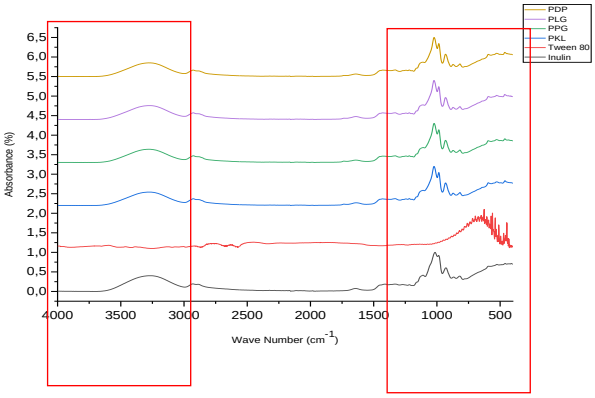
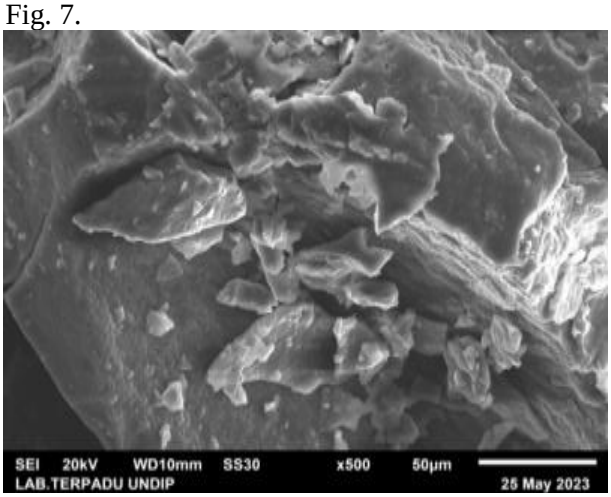


Fig. 6 IR spectrum extract and encapsule (Developed by the authors)

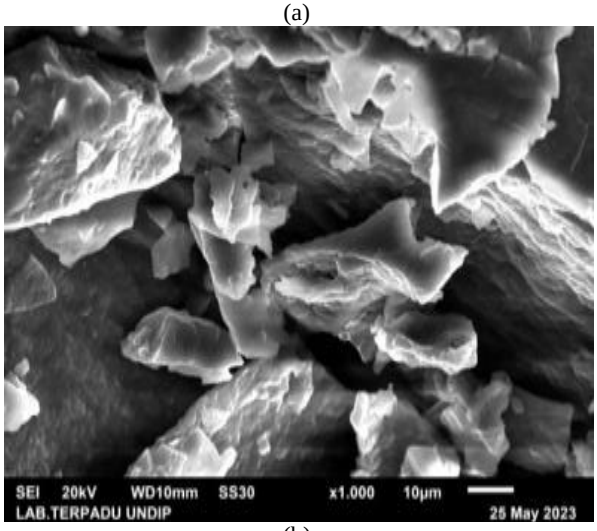
Peaks appeared at wavelengths of 2850-2970 and 1340-1470 cm^{-1}, which may indicate the presence of C-H alkane functional groups that usually appear at these wavelengths. At wavelengths of 675-995 cm^{-1} peaks appear which may indicate the presence of C-H alkene functional groups that usually appear at wavelengths of 3010-3095 and 675-995cm^{-1}. The peak at wavelengths 690-900 cm^{-1} which likely indicates the presence of the C-H functional group of the aromatic ring that usually appears at wavelengths of 3010-3100 and 690-900cm^{-1}. Peaks appeared at wavelengths 3200-3600cm^{-1} which may indicate the presence of functional groups of O-H hydroxy/phenol bond alcohols that usually appear at these wavelengths [25]. Peaks appear at wavelengths of 1610-1680 cm^{-1} which may indicate the presence of C=C alkene functional groups that usually appear at these wavelengths. Peaks appear at wavelengths of 1180-1360 cm^{-1} which may indicate the presence of Amine/Amide C-N functional groups that usually appear at these wavelengths. Peaks appear at wavelengths of 1050-1300 cm^{-1} which may indicate the presence of C-O functional groups of alcohols/ethers/carboxylic acids/esters that usually appear at those wavelengths.

The inulin spectrum shows a wide absorption band at wavenumbers 3200-3600 cm-1 which indicates an O-H (hydroxyl) bond stretch and wavenumbers 2850-2970 cm^{-1} and 1340-1470 cm^{-1} indicate C-H bond stretching. The IR spectrum in inulin has a wide absorption at wavenumber 3395 cm-1, which indicates an O-H (hydroxyl) bond stretch, which is the main characteristic of inulin; the wavenumber 2930 cm^{-1} indicates a C-H bond stretch, while the red section indicates a cross-linked polymerization reaction of the extract with the coating [26]. The results of the comparison of the IR spectra between the coating and the encapsulation results show that encapsulation is achieved, and it can be seen that all the spectra present in the encapsulation material appear on the encapsulation.

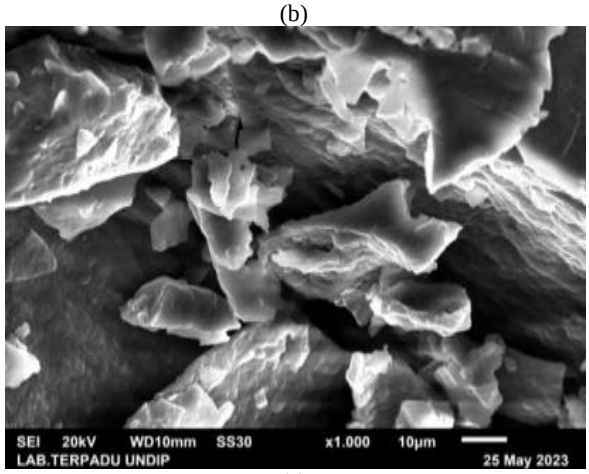
Scanning Electron Microscopy was used to observe the morphological structure of the encapsulated PKL at magnifications of 500, 1000, 2500, and 5000, as shown in Fig. 7.



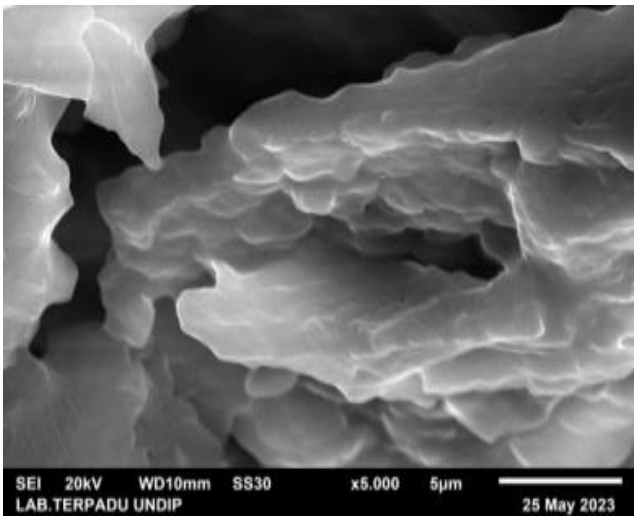
(a)



(b)



(c)



(d)

Fig. 7 The encapsule morphology analysis results: (a) 500x, (b) 1,000x, (c) 2,500x, (d) 5,000x (Developed by the authors)

Using coating materials, such as inulin, in the encapsulation process produces particles of various sizes, irregular surface structures, and deep hollows in the walls. Encapsulants must have a smooth and homogeneous surface with a slightly rounded shape and few wall folds and cracks because encapsulants with rough surfaces are more sensitive to oxidation reactions than smooth surfaces. A good microcapsule is round without wrinkles, which means that the active ingredients are very well encapsulated [27, 28].

3.8. Bioavailability of In Vitro Encapsulants

In vitro encapsulant bioavailability test using pH buffer 1.2 (gastric pH simulation) and pH buffer 7.4 (blood pH simulation) with time variation for 30; 60; 120 and 240 min. The bioavailability test was only carried out on the ethanol extract encapsulant of Kampung Laut perepat leaves because it had the highest yield, water content, solubility, and total phenol, flavonoid, and antioxidant test results. An in vitro bioavailability test was performed with an encapsulated ethanol extract from perepat leaves inserted into the shell of a capsule weighing 500 mg. The test results are listed in Table 4.

Table 4 % bioavailability of encapsules in vitro (The authors)

Time Variations (min)	Absorbance		Concentration (ppm)		% Encapsulant Release	
	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4
30	2.764	3.155	174.437	454.571	34.887	90.914
60	2.977	3.296	187.918	474.714	37.584	94.943
120	3.280	3.328	207.095	479.286	41.419	95.857
240		3.422		492.714		98.543

Bioavailability in vitro encapsulation of ethanol extract from *S. alba* leaves with inulin coating from the PKL, at a pH 1.2 min 30 there showed a release of approximately 34.887%, and in the 60 min, it rose to 37.584%, and in 120 min, it rose again to 41.419%. Furthermore, at pH 7.4, in the 30th minute, there was a release reaching 90.91%; in the 60th minute, there was a release reaching 94.94%; at 120 min, there was a release reaching 95.85%; and at 240 min, there was a release of 98.54%. The bioavailability of phenolic compounds is influenced by various factors, as they must pass through several membranes and be exposed to various metabolic processes. In addition, each class or subclass of phenolic compound has a different metabolic fate. Solubility is a physicochemical property of phenolic compounds that significantly affects bioavailability.

Low water solubility, absorption, and rapid metabolism lead to the low bioavailability of phenolic compounds [29]. Inulin is a polysaccharide encapsulation material that binds the core material (active component) into amorphous solid units, thus allowing the formation of a solid and stable capsule [30]. In addition, when the objective is to ensure the transport of bioactive substances and their release in certain parts of the digestive system, polysaccharides stand out because most of them protect against the breakdown of encapsulation in the stomach and upper gastrointestinal tract, and are broken down into the colon where most of the bioactive substances are absorbed in the blood.

4. Conclusion

The highest encapsulation efficiency was observed in PPG samples and the lowest in PKL. The IR spectrum shows the presence of C-H Alkane functional groups, C-H Alkene functional groups, C-H aromatic ring functional groups, O-H functional groups, hydrogen/phenol bond alcohols, C=C alkene functional groups, and C-O All alcohol/ether/carboxylic acid/ester functional groups. The IR spectrum confirms the cross-linking reaction and the formation of the Encapsule. PKL has the highest TPC and TFC, as well as antioxidant activity, for extraction and encapsulation. *In vitro* bioavailability of encapsulated PKL at pH 1.2, which has the largest encapsulated release within 120 min, while at pH 7.4, which has the largest encapsulant release, was 240 min.

Author Contributions

Y and AN contributed to the design and implementation of the research, H, FT, and ILT to the analysis of the results and to the writing of the manuscript. ML and AMD conceived the original and supervised the project.

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