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Nanoencapsulation of Simpur (Dillenia indica L.) Leaf Extract For Antibacterial

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Abstract. Simpur leaf (*Dillenia indica* L.) as a traditional medicinal plant spread in the Bangka Belitung Islands. Secondary metabolites contained in simpur leaf extract are alkaloids, flavonoids, phenols, tannins, saponins, steroids, and terpenoids. Compounds that provide pharmacological properties such as polyphenols are unstable to the influence of temperature and high light intensity so that they are easily oxidized. The challenge to protect the damage of these compounds can be done by means of nanoencapsulation. This study aims to determine the size and efficiency of the nanoencapsulation of simpur leaf extract and its antibacterial bioactivity against *Staphylococcus aureus* and *Escherichia coli* bacteria. Manufacture of nanoencapsulation using the nanoprecipitation method with the constituent components of PCL (1.5 g), Tween 80 (50 mL), simpur leaf extract (0.15 g; 0.25 g; 0.35 g). Antibacterial activity testing using disc diffusion method. Nanoencapsulated extract mass 0.15 g; 0.25 g; and 0.35 g have sizes of 167.2 nm, respectively; 208.7 nm; and 229.1 nm and encapsulation efficiency of 88.21%; 56.77%; and 5.34%. The antibacterial activity of the nanoencapsulation and extract was more effective in inhibiting the growth of *Staphylococcus aureus* bacteria than *Escherichia coli* bacteria. Strength of activity against *Staphylococcus aureus* bacteria in nanoencapsulated extracts was categorized as moderate to strong and *Escherichia coli* bacteria in extracts were categorized as moderate.

Keywords : Antibacterial, Simpur leaf (*Dillenia indica* L.), nanoencapsulation

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Introduction

Infection is a type of disease caused by pathogenic bacteria that enter the body, multiply and cause disease. One of the bacteria that often causes infection is *Staphylococcus aureus* and *Escherichia coli* [1]. Treatment due to infection by bacteria requires treatment with antibiotics. Traditionally, simpur leaves are used by the Malays in the province of Bangka Belitung for the treatment of high blood pressure [2]. Simpur leaves can be used as herbal medicine [3].

Based on research that has been done, simpur leaves have pharmacological effects as antidiabetic, antioxidant, and antimicrobial activity [4]. Secondary metabolites of simpur leaf ethanol extract contain alkaloids, phenols, flavonoids, steroids, terpenoids, tannins and saponins [5]. Research that has been done states that simpur leaves contain terpenoids and flavonoids [6]. The content of alkaloids and flavonoids in a material acts as an inhibitor for the growth of pathogenic bacteria [7]. According to research [6] that have been carried out have shown that simpur leaves have antibacterial activity. Compounds that function in providing pharmacological effects have weaknesses, namely they are not stable to the effects of temperature and high light intensity so that they are easily oxidized, such as polyphenol compounds [8]. The challenge that needs to be done to protect or protect these compounds from existing damage is by means of nanoencapsulation.

Nanoencapsulation can coat the core in the form of an active compound (in the form of liquid, solid, or gas) with a certain polymer. Nanoencapsulation in small size has the advantage of protecting a compound from decomposition, adverse environmental influences such as oxidation damage, hydrolysis, and heat degradation as well as regulating the release of active compounds [9]. The ideal encapsulation material is a polymer that is non-toxic, does not react with the core material, is biodegradable, and can form a good layer formation [10]. The nanocapsule method can increase bioactivity as an antibacterial to protect the active ingredients. The effect of the encapsulated material depends on the characteristics of the protected core which includes the bioactive ingredients contained therein [11].

In this study using a coating that is polycaprolactone (PCL). PCL has complex drug permeability and mechanical resistance. The results of previous studies conducted by [12] using polycaprolactone that polycaprolactone coating material is more effective in protecting the active ingredient than other coating materials with the obtained encapsulation efficiency of 72.69 to 99.99%. Encapsulation efficiency will increase with the addition of tween 80 which will create good stability and texture [13]. Therefore, in this study, we will examine the nanoencapsulation of simpur leaf extract (*Dillenia indica* L.) for antibacterial which has never been done before.

Experimental

Materials

The materials used in this study were simpur leaf fine powder, acetone, concentrated H_2SO_4, glacial acetic acid, distilled water, 1% $FeCl_3$ and 5% $FeCl_3$, concentrated HCl, Dragendorf reagent, Mayer's reagent, Wagner's reagent, Mg metal powder, polycaprolactone (PCL), chloroform, tween 80, nutrient broth (NB), nutrient agar (NA), amoxicillin, Dimethyl Sulfoxide (DMSO), *Escherichia coli* bacteria, and *Staphylococcus aureus* bacteria.

Equipment used were blender, Pioneer TM analytical balance, jar, 50 mL Erlenmeyer Iwaki, 100 mL Iwaki volumetric flask, 50 mL Iwaki beaker, 10 mL measuring cup, 100 mesh sieve, petri dish, test tube, filter paper, rotary evaporator, homogenizer, magnetic stirer, disc paper, aluminum foil, Bunsen, tween 80, buncher funnel, ose needle, Particle Size Analyzer (PSA), FTIR spectrophotometer, UV-Vis spectrophotometer, Laminar Air Flow (LAF), hot plate, Hirayama HVA-110 autoclave, caliper, and the Memmert INB 500 Microbial incubator.

Sample Preparation

Simpur leaves were obtained from Namang Village, Koba District, Central Bangka Regency. Simpur leaves are dried for 15 days by airing without contact with direct sunlight. Then, the dried leaves were mashed using a blender until a fine powder was obtained and filtered through a 100 mesh sieve.

Simpur Leaf Extract

Simpur leaf powder that has been mashed is taken as much as 250 g. After that, it was macerated using 2.5 L of acetone solvent by soaking it for 3x24 hours with stirring every 24 hours at room temperature. The extract obtained was filtered using vacuum filtration. Then it is concentrated using a rotary evaporator at a temperature of 56oC until a concentrated extract is obtained and then weighed and the yield value is calculated using the following formula:

% Yield = Weight of Extract/Weight of Simplicia x 100% [5].

Phytochemical Screening

The acetone extract of simpur leaves was tested qualitatively against secondary metabolites of alkaloids, phenols, flavonoids, steroids/ terpenoids, tannins, and saponins.

Nanoencapsulated Formulation

Variations in the mass of simpur leaf extract were made at 0.15 g; 0.25 g; 0.35 g. A total of 1.5 grams of PCL was dissolved in 15 mL of chloroform and then mixed with simpur leaf extract and 50 mL of 0.5% tween 80 solution. then, in the homogenizer for 1 hour at 4500 rpm. After that, the solution was put into a spray bottle and sprayed into 250 mL of distilled water for 1 hour. Next, the solution was filtered and dried at room temperature for one night [14].

Encapsulation Efficiency (%)

Encapsulation efficiency was determined using a UV-Vis spectrophotometer at a wavelength of 665 nm by making a standard curve of simpur leaf extract based on its absorbance value at a concentration of 5; 10; 15; 20; 25 mg/10 mL of distilled water. Before dissolving with distilled water, 2 drops of simpur leaf extract were added. Next, 0.1 g of the nanoencapsulated sample was dissolved in 10 mL of distilled water and the absorbance value was measured at 665 nm [15]. Encapsulation efficiency is calculated using the following formula:

EE% = w1 - w2/w1 x 100%

Note:

w1= amount of extract contained in encapsulation (mg)

w2 =amount of extract used in encapsulation (mg)

Antibacterial Activity Test

A total of 3 g of nutrient agar (NA) and 1.6 g of nutrient broth (NB) were added to 200 mL of distilled water. Then, heat it until it boils and dissolves. Petri dishes were coated with paper and put in heat-resistant plastic and sterilized using an autoclave at 121^{o}C for 15 minutes. Rejuvenation of bacteria by inserting 5mL of sterilized agar medium into a petri dish until it is covered with the bottom of the cup then wait for it to solidify. After solidifying, take a needle of bacteria and then scrape it onto the media in a zig-zag fashion. Incubate at 37^{o}C for 24 hours in an incubator. Preparation The test solution was made with variations in the mass of extract and nanoencapsulation, namely 0.15 g; 0.25 g; and 0.35 g dissolved in 1 mL DMSO. Then, put a 6 mm diameter paper disc into the test solution. The disc paper in the test solution is inserted into the media that has been smeared with bacteria first. Incubate at 37^{o}C for 24 hours in an incubator and measure the antibacterial inhibition zone using a caliper [16]. The formula for the antibacterial zone of inhibition is as follows:

Inhibition Zone = Clear zone diameter (mm) – Disc paper diameter (mm).

Results and Discussion

Phytochemical Screening

The simpur leaf acetone extract that has been obtained was subjected to phytochemical screening to determine the secondary metabolites contained in the simpur leaf acetone extract showed positive results of secondary metabolites, namely alkaloids, phenols, flavonoids, steroids/ terpenoids, tannins and saponins. The results of the phytochemical test of simpur leaf extract can be seen in Table 1.

FTIR Analysis of Simpur Leaf Extract

FTIR analysis was carried out on simpur leaf extract aimed to determine the functional groups of secondary metabolites contained in simpur leaf extract seen from the spectrum and ab-

Table 1. Simpur Leaf Extract Phytochemical Test Results

Phytochemical Test	Observation	Results
Mayer Alkaloids	A White precipitate is formed	+
Dragendroff Alkaloids	An orange precipitate is formed	+
Wagner Alkaloids	Formation of reddish brown color	+
Phenol	Formed blackish green color	+
Flavonoids	It is dark red and has foam	+
Steroids	Blue green color	+
Terpenoids	Formation of red-brown color	+
Tannins	Formed black bluish / green	+
Saponins	Foam formed	+

sorption band as wave numbers. The results of the FTIR analysis of Simpur Leaf Extract can be seen in Table 2.

Based on Table 2, the results of the FTIR analysis showed the presence of flavonoids in the form of O-H groups, aliphatic C-H groups, C=O groups, and C-O groups. The presence of alkaloids is suspected to be a C=N group, a C-N group. Phenolic compounds are strengthened by the presence of an aromatic C=C group. The $-CH_2-$ and $-CH_3-$ groups indicate the presence of a geminal dimethyl group $-CH(CH_3)_2$ as a characteristic of steroids/terpenoids. The presence of tannins is suspected to be an O-H group, an aliphatic C-H group, and an aromatic C=C group. The presence of saponins in the form of an O-H group and an aromatic C=C group [17].

Simpur Leaf Extract Nanoencapsulation

Nanoencapsulation of simpur leaf extract in this study used the nanoprecipitation method. The nanoencapsulation process was carried out by PCL emulsification, simpur leaf extract, and tween 80 at a concentration of 0.5%. The use of PCL as a synthetic polymer coating that can control the release of active substances and as a component of the encapsulation wall to protect the extract. PCL which is hydrophobic but easily soluble in volatile solvents such as chloroform. Chloroform was used to dissolve PCL. According to research conducted by [14]

PCL coating is effective in the encapsulation process which greatly affects the release of active ingredients with a longer time in nanocapsules. Differences in mass variation of simpur leaf extract in formula A; formula B; and formula C can affect the size and efficiency of the resulting encapsulation. Emulsifier tween 80 is used as an emulsifier to stabilize the emulsion formed for the encapsulation process [18]. Research [18] showed that tween 80 concentration of 0.5% resulted in the highest encapsulation efficiency at PCL of 43.5%.

Dispersion of an emulsion solution in which there is droplet formation. The emulsion

Table 2. Results of FTIR Analysis of Simpur Leaf Extract

Wave Number (cm^{-1})	Reference [17][25]	Functional groups
3395	3200-3600	O-H
2926	2850-2970	C-H aliphatic
1698	1685-1710	C=O
1611	1600-1680	C=C aromatic
1527	1500-1650	C=N
1449	1450-1375	$-CH_2-$
1376	1340-1470	$-CH_3$
1233	1180-1260	C-N
1033	1000-1060	C-O

solution formed is dispersed to determine the size of the encapsulation by spraying so that the capsules are less agglomerated and prevent phase separation. This spraying makes the organic solvent chloroform diffuse rapidly into the water and will evaporate. The stirring of the magnetic stirrer solvent will be stopped, it will be seen that the encapsulated formed descends to the bottom of the container. Droplets that are in solution for a long time can cause low encapsulation efficiency. Drying of nanocapsules in the form of powder will be analyzed for particle size in the form of PSA test and analysis of encapsulation efficiency [14]. Illustration of nanoencapsulation can be seen in Figure 1.

Based on Figure 1 on the nanoencapsulation system, it shows that the nanocapsule has a head or outer part in the form of an ether group from PCL and a tail part in the form of an ester group surrounded by tween 80. The head is hydrophilic and the tail is hydrophobic from PCL will interact with tween 80 to form a capsule. which is stable to protect the core material in the form of simpur leaf extract which is hydrophobic where the core material will not react with the coating material so that the activity of the core material in it will be the same as before nanoencapsulation [20].

PSA Analysis Nanoencapsulated Simpur Leaf Extract

PSA analysis was carried out using the Particle Size Analyzer (PSA) instrument which aims to determine the size of nanoencapsulated particles in simpur leaf extract. The results of PSA analysis can be seen in Table 3.

Based on the results of the analysis, Table 3 shows that the size of the nanocapsules of simpur leaf extract is in accordance with the literature of [21] that the nanocapsule size is 100-500 nm. The higher the mass of the extract sample, the larger the particle size obtained. This is because the particles formed from polymer decomposition on the surface of the extract globules are very large and dense so that they clump together to form aggregates into larger particle sizes and

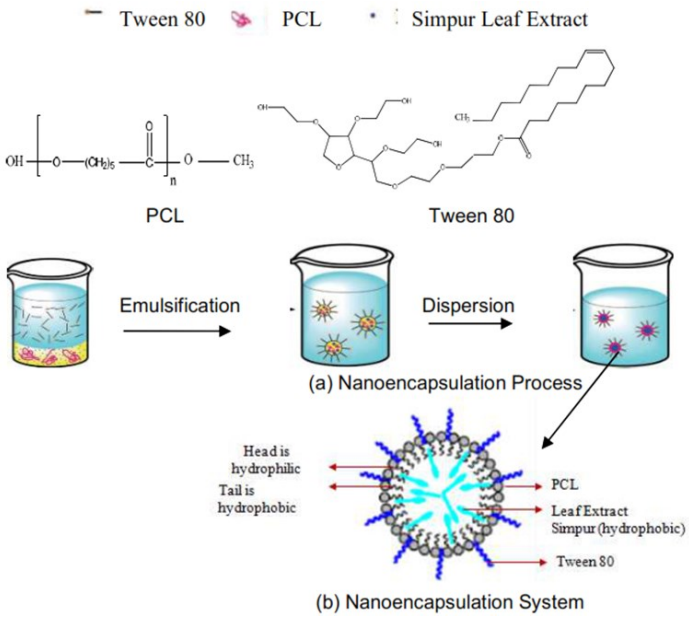

Figure 1. Illustration (a) Nanoencapsulation Process (b) Nanoencapsulation System [20].

Table 3. Results of Nanoencapsulated PSA Analysis Simpur Leaf Extract

Formula	Diameter (nm)	PI	Nanocapsule Size (nm)
A	167.2	0.424	
B	208.7	0.446	100- 500
C	229.1	0.368	

are difficult to break down into smaller particle sizes [22]. The value of the polydispersity index (PI) is the particle size distribution. According to previous research [23] if the polydispersity index (PI) <0.5 indicates a more uniform particle size, while the polydispersity index (PI) >0.5 indicates the particle size tends to be non-uniform. In this study, the three PI values of simpur leaf extract were obtained, namely the polydispersity index (PI) <0.5 that the nanocapsules were more uniform.

Simpur Leaf Extract Efficiency

The encapsulation efficiency of simpur leaf extract shows the amount of extract that is coated in the nanoencapsulation. Encapsulation efficiency was measured using a UV-Vis spectrophotometer by making a standard curve based on the absorbance value using the maximum wavelength at 665 nm [15]. The results obtained in Table 4.

Based on Table 4, the results obtained from the encapsulation efficiency that the higher the mass of simpur leaf extract in nanoencapsulation, the smaller the encapsulation efficiency value. Increasing the mass of the extract will decrease the encapsulation efficiency. This was due to an increase in the mass of the extract where the polymer coating was no longer able to protect the extract and maintain the retention of the extract, resulting in the extract coming out of the nanoencapsulation [22]. The larger the size of the nanocapsule, the decrease in the efficiency value is caused by the smaller surface area of the nanocapsule so that the polymer coating that is able to protect the extract is getting smaller [24].

Table 4. Results of Encapsulation Efficiency (EE%)

Formula	Nanocapsule Size (nm)	EE (%)
A	167.2	88.21
B	208.7	56.77
C	229.1	5.34

FTIR Analysis of Simpur Leaf Extract Nanoencapsulation

FTIR analysis was carried out to determine the functional groups contained in the nanoencapsulation of simpur leaf extract indicated the presence of absorption bands and spectral shapes at certain wave numbers. The results of nanoencapsulation FTIR analysis of simpur leaf extracts can be seen in Table 4.5.

Based on Table 5 in formula A; formula B; and formula C shows almost the same functional groups as PCL and tween 80 are found at wave numbers 3443.05-3442.91 cm-1 in the form of O-H groups, wave numbers 2923.37-2924.68 cm-1 in

Table 5. Results of FTIR Nanoencapsulation Analysis of Simpur Leaf Extract

	Wavenumber (cm^{-1})				
Functional Groups	Formula A	Formula B	Formula C	Simpur Leaf Extract	Reference [17] [25]
O-H	3443.05	3442.07	3442.91	3395.92	3330-3500
C-H	2924.68	2923.37	2924.57	2926.86	2840-3000
C=O ester	1733.01	1733.46	1733.13	-	1700-1740
C=O carboxyl	-	-	-	1698.77	1660-1750
C=C	1637.70	1636.97	1637.25	1611.65	1600-1680
$-CH_2-$	-	-	-	1449.00	1450-1375
$-CH_3$	-	-	-	1376.68	1340-1470
C-O ether	1098.30	1102.26	1098.96	-	1085-1300
C-O alcohol	-	-	-	1033.79	1000-1060
C-N	-	-	-	1233.52	1180-1360

the form of C-H alkane bonds, wave number 1733.01-1733.46 cm-1 is a C=O ester group, wave number 1098.30-1102.26 cm-1 is a C-O ether group. Wave number 1636.97-1637.70 cm-1 in the form of aliphatic C=C group with tween 80. Nanoencapsulation of simpur leaf extract indicated by a shift in the wave number in the extract and nanoencapsulation from wave number 3395.92 cm-1 to 3443.05- 3442.91 cm-1 in the form of an O-H group, the wave number shifted from 2926.86 cm-1 to 2923.37-2924.68 cm-1 in the form of a C-H group and the wave number shifted from 1611.65 cm-1 to 1636.97- 1637.70 cm-1 in the form of a C=C group. This is due to the interaction between functional groups and nanoencapsulation. The emergence of two new peaks in formula A; formula B; and formula C in the form of functional group C=O ester at wave number 1733.01-1733.46 cm-1 and the emergence of a new peak in the form of C-O ether group at wave number 1098.30-1102.26 cm-1, which is a characteristic of the functional group of PCL is an aliphatic polyester and tween 80 is a polyoxyethylene fatty acid ester. The results obtained that the larger the mass of the extract in the nanoencapsulation caused the formation of a high intensity and a lot closer to the intensity of the extract [25].

Antibacterial Activity Test

The method used in antibacterial testing is the disc diffusion method. The diffusion method aims to determine the sensitivity or antibacterial strength to an antibiotic by measuring the diameter of the clear zone formed around the paper disc [26]. This antibacterial test uses gram-positive bacteria, namely *Staphylococcus aureus* and gram-negative bacteria, namely *Escherichia coli*. The graph of the inhibition zone of the extract and the nanoencapsulation of the extract against S. *aureus* and *E. coli* bacteria can be seen in Figure 2.

Based on Figure 2. The results obtained were that the antibacterial activity of simpur leaf extract against *S. aureus* was stronger than *E. coli* bacteria. Criteria for antibacterial strength : > 20 mm is very strong inhibition; 10-20 mm is strong inhibition; 5-10 mm is moderate inhibition; and 0-5 mm is weak inhibition [26]. According to [27] this is because *S. aureus*, including gram-positive bacteria, has a cell wall consisting of several layers of peptidoglycan forming a thick and rigid structure where the compound more easily enters the bacterial cell wall. Meanwhile, *E.coli* is a gram-negative bacterium that has a cell wall in the form of a peptidoglycan layer forming a thin structure so that it has low sensitivity to antibacterial activity. The antibacterial activity of nanoencapsulated

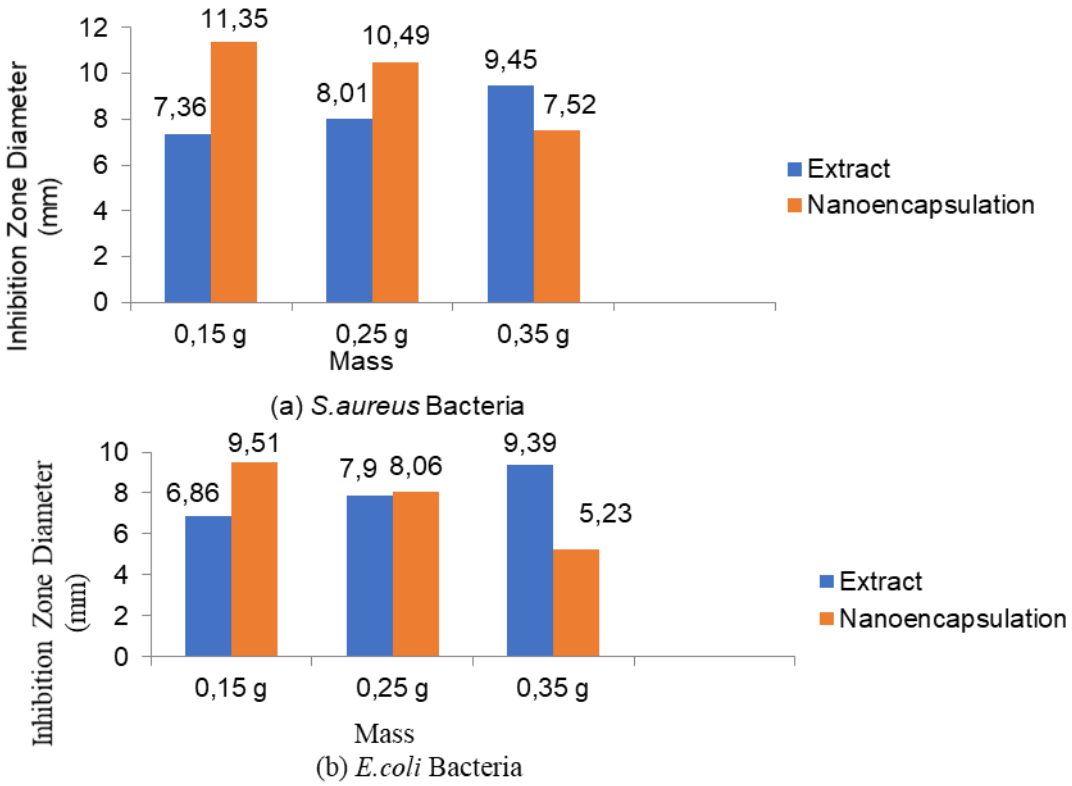

Figure 2. Graph of Extraction Inhibition Zone and Extract Nanoencapsulation (a) *S. aureus* bacteria and (b) *E. coli* bacteria

simpur leaf extract was stronger than the extract alone and was more effective at inhibiting the growth of *S. aureus* than *E. coli* bacteria. This is because the influence of the larger nanocapsule size on the mass of the nanoencapsulated extract causes its antibacterial activity to be weaker [28]. The small size of the nanocapsule has a larger surface contact area which can increase the amount and solubility of more active substances so that the antibacterial activity is stronger [29]. The small size of the nanocapsule encourages the destruction of the bacterial cell wall [30].

Based on Figure 2 shows the resulting inhibition zone against S. aureus and E. coli bacteria in formula C with a mass of 0.35 g with an extract mass of 0.35 g which is greater in formula C nanoencapsulated than the extract. This result is different from the literature which shows that the antibacterial activity of the nanoencapsulated is stronger than that of the extract. This is because the large nanocapsule size causes a small surface area so that the polymer coating that is able to protect the extract in the nanoencapsulation is less than the extract alone and during the nanoencapsulation process there are external environmental influences such as temperature, light, and air that can affect stability so that the bioactive content of nanocapsules is less. from the extract [28]. The results of the antibacterial inhibition of the extract and nanocapsules of simpur leaf extract can be seen in Figures 3 and 4. Illustration of nanoencapsulation against bacteria can be seen in

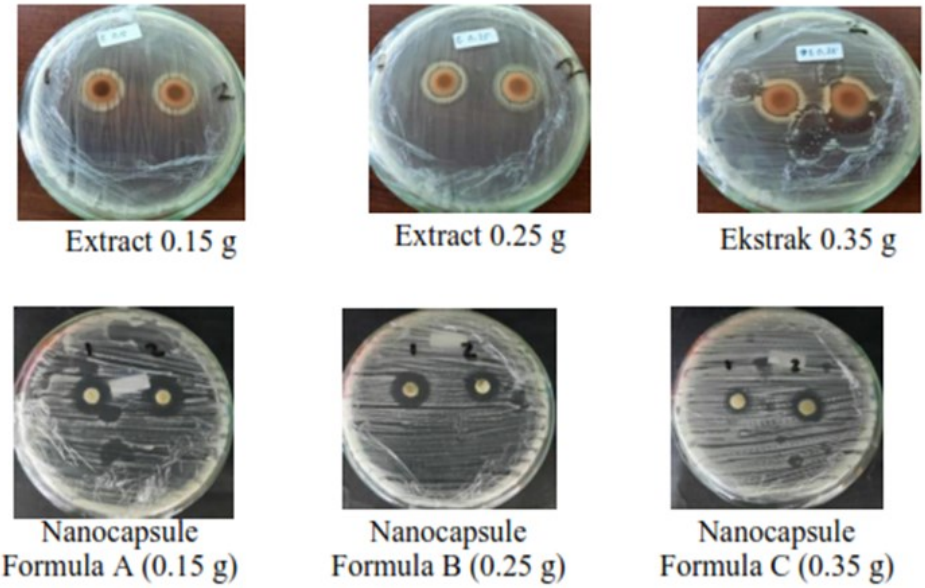

Figure 3. Inhibition of simpur leaf extract and nanocapsules against S. Aureus

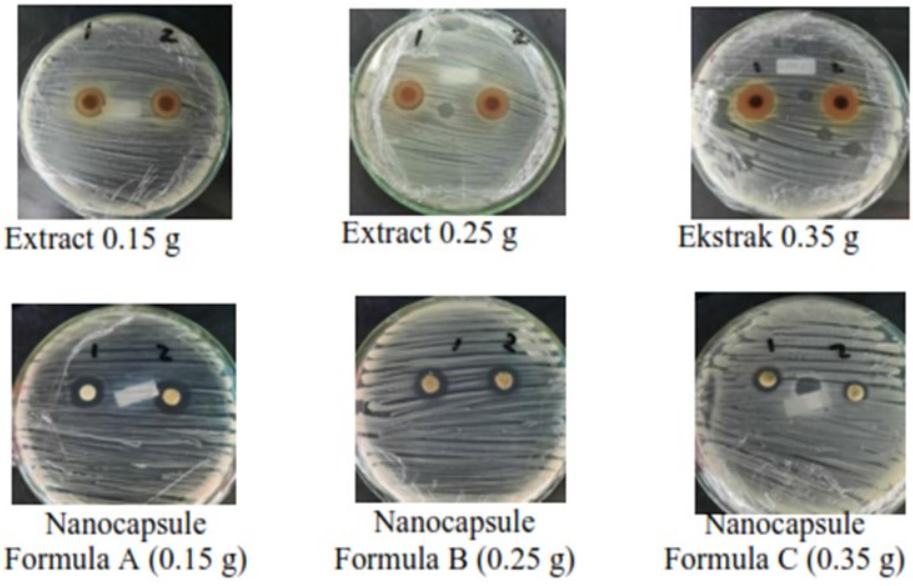

Figure 4. Inhibition of simpur leaf extract and nanocapsules against E. coli

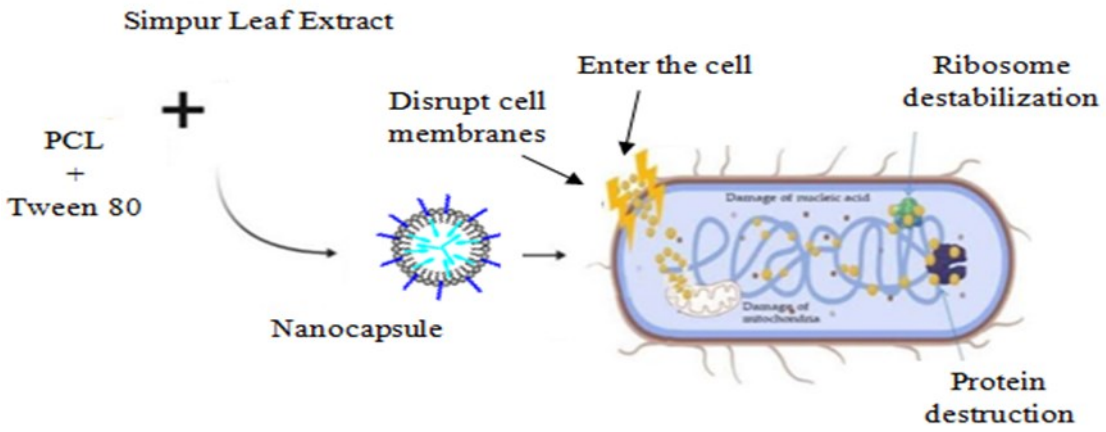

Figure 5.

Based on Figure 5, it shows that PCL interacting with tween 80 on the outside will protect the extract on the inside to form nanocapsules that work as antibacterial by damaging the cell wall so that it will disrupt and damage cell membranes and cause disruption of cell metabolic activity and even cell death [31].

The diameter of the inhibition zone for the positive control in S. aureus was 26.07 mm which was categorized as very strong and 21.10 mm in E.coli was categorized as very strong. The positive control used in the second antibacterial activity test was amoxicillin. Amoxicillin was used as a positive control because amoxicillin is a broad-spectrum penicillin derivative used to treat various bacterial infections. Amoxicillin will inhibit bacterial growth by inhibiting bacterial cell wall synthesis by binding to one or more amoxicillin-protein bonds [32]. The negative control in the activity test of these two antibacterials was DMSO. The results of the inhibition zone on the negative control against S. aureus and E. coli bacteria were 0 mm which indicated that the results of the antibacterial test of the sample were not affected by DMSO.

The antibacterial activity of simpur leaf extract contains secondary metabolites, namely alkaloids, flavonoids, phenols, tannins, and saponins which are polar while steroids and terpenoids are non-polar. The mechanism of alkaloids is antibacterial which can interfere with the peptidoglycan constituent components in bacterial cells by damaging the cell membrane which causes cell death [29]. Secondary metabolites of flavonoids are able to damage cell walls and interfere with metabolic processes that cause damage to the permeability of bacterial cell walls [33]. Phenolic compounds act as antibacterial by damaging cell membranes, inactivating enzymes and denaturing proteins so that cell walls are damaged. The mechanism of action of tannins as antibacterial can interfere with peptidoglycan synthesis so that the formation of bacterial cell walls is less than perfect and bacterial cells will die [34]. The mechanism of saponin compounds as antibacterial by lowering the surface tension of the cell wall causes leakage of proteins and enzymes from within the cell [35]. The antibacterial ability of steroids by damaging the bacterial cell membrane. The terpenoid mechanism involves membrane damage by lipophilic compounds [36].

Conclusion

The results of this study can be concluded that the nanoencapsulation size of simpur leaf extract in formula A; formula B; and formula C were obtained respectively at 167.2 nm; 208.7 nm; and 229.1 nm with encapsulation efficiency of 88.21%; 56.77%; and 5.34%. The nanoencapsulation of simpur leaf extract had stronger bacterial activity than the extract alone and the nanoencapsulation of the extract was more effective at inhibiting S. aureus than E. coli bacteria which obtained an inhibition zone in the mass variation of formula A 0.15 g; formula B 0.25 g; and formula C 0.35 g of 11.35 mm, respectively; 10.49 mm; 7.52 mm and 9.51 mm; 8.06 mm; 5.23mm. The greater the mass of the extract in the nanoencapsulation and the larger the size of the nanocapsule, the smaller the antibacterial activity. Suggestions that need to be done for further research by making mass variations on polymer coatings and concentration variations on surfactant tween 80 in order to obtain maximum results on encapsulation size and efficiency as well as stability tests and other biologi-

cal activity tests.

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