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Research Article

Microencapsulation of Kaffir Lime Peel (*Citrus Hystrix DC*) Essential Oil Using Chitosan and Maltodextrin as Matrices on Freeze Drying Process As AntiCellulitis

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Abstract: Research has been conducted to microencapsulate kaffir lime (*citrus hystrix dc*) essential oil (KLEO). This microcapsule products was formed using chitosan and maltodextrin as matrices on freeze drying process. The microencapsulated KLEO can act as antibacterial of *Staphylococcus aureus* cause of cellulitis Cellulitis is an infectious disease that occurs in the skin that occurs in people with normal immunity. The main cause of cellulitis is gram-positive bacteria such as *Staphyloccocus aureus*. Kaffir lime peel contains various compounds that can be used, one of which is the antibacterial *Staphylococcus aureus* that causes cellulitis. The purpose of this study was to microencapsulate the essential oil of kaffir lime peel using the freeze drying method as an anti-cellulitis. The freeze drying method has the advantage of maintaining the quality of the dried product. This microencapsulation process is carried out using chitosan and maltodextrin coatings which are a combination of coatings that are effective against essential oil microencapsulation so as to maintain the core compound of kaffir lime peel essential oil. Then the particle size of the microencapsulated was 1.72 µm and oval in shape. The results of the bacterial test is the clear zone that was obtained was the best for *Staphylococcus aureus* bacteria at a variation of 0.1 grams of chitosan: 5.9 grams of maltodextrin, which was 22.5 mm. It can be concluded that the kaffir lime peel essential oil microencapsulation has a good prospect as an anticellulitis powder preparation compared to the kaffir lime peel essential oil extract without the microencapsulation process. This research can also be used as an effort to overcome kaffir lime peel waste which cannot be used in general. In the future, it is hoped that it will develop into an anti-cellulitis dosage form that can be mass-produced.

Keywords: Bacteria, Cellulitis, Coating, Freeze drying, Microencapsulation

Introduction

Encapsulation is a process in which core materials are eclosed within the confines of a coating material, most often a molecular container [6]. Utilizing microencapsulation technology to achieve this goal of controlled release is one of the most effective methods thus far [7]. However, at present, there is inadequate study and research related to the technology to encapsulate volatile materials in microcapsules[8]. Encapsulation process has several advantages, including improve the solubility, dispersion ability and flow properties of the core material, reducing the nature of the initiation of the core material, protection of core or unstable material from environmental influences before used, increased shelf life by preventing degradation reactions (oxidation, dehydration), and control release of core material[9].

Two type of encapsulation has been performed in several research. Nanoencalsulation is the coating of various subtances within another material at sizes on the nano scale. Microencapsulation is similar to nanoencapsulation aside from it involving larger particles and having been done for a greater

period of time than nanoencapsulation[12]. The primary reason for microencapsulation is found to be either for sustained or prologue drug release such as main compound of essential oil. The drugs which are sensitive to moisture light and oxygen also volatile in nature and vaporize at room temperature can be protected by microencapsulation[16].

Cellulitis is an infectious disease that occurs in the skin that occurs in people with normal immunity and can affect children to old age. Clinical symptoms that appear are erythema, pain, edema, inflammatory suppuration, fever, chills, and local pain. The main cause of cellulitis is gram-positive bacteria such as *Staphylococcus aureus* [1].In Indonesia there are more than 150,000 cases per year according to the Indonesian Doctors Association. Cellulitis is common in men and the most common location is in the lower extremities. Delay in treatment can lead to disability due to tissue necrosis or even death from sepsis. Information on the epidemiology of cellulitis in Indonesia is still very limited. [2]. Most cases of cellulitis are caused by bacteria *Staphylococcus aureus* that enter from wounds on the skin such as surgical wounds and scrapes [3]. Epidemiological studies show that infection due to *Staphylococcus aureus* in the world has increased in the last two decades [4]. In overcoming cellulitis treatment using antibiotics is commonplace, but too often taking antibiotics can have a negative impact on health. This makes it important to develop an appropriate method using essential oil microencapsulation as an anticellulitis[18].

Comparative studies of the microcapsules from basil oil obtained from steam distillation of basil leaves, showed a potential source of anti bacterial activities. The process of encapsulation used freeze-drying method[20]. The characterization of microcapsule was examined by means of SEM. The GC-MS analyzed showed the component of basil oil such as Ecitral (32.93%), Z-citral (23.96%), linalool, isocaryophyllene, α-humulent and caryophyllene oxide[15]. The results showed that microcapsules of basil oil had the best controlled release in the sample of variation C (maltodextrin : β-cyclodextrin = 1:2). The physics properties of microcapsules from basil oil was dust white solids, not agglomerate and had scent like typical basil. The results of SEM characterization showed average size of microcapsule that was < 2 μm[13].

Similar to above finding, kaffir lime (*Citrus hystrix DC*) shows the same potential results. But mostly, the main utility of kaffir lime was focusing on leaves, and so peel of this kind of fruit was very rare. The kaffir lime fruit is often not utilized so that it becomes kaffir lime peel waste. Kaffir lime peel contains the main components of -pinene (35.89%), citronellal (12.31%), limonene (14.95%) and terpinene-4-ol (3.91%). It is known to have antibacterial ability because the compound -pinene has been shown to have an antibacterial effect by inhibiting the synthesis of DNA, RNA polysaccharide walls and cell membrane ergosterol [5]. This research aims to make a microencapsulation of kaffir lime peel essential oil which functions as an anticellulitis powder. One of the properties of essential oils is that they are volatile, so they need to be made into a microencapsulated form so that the active compounds of essential oils are maintained. It is necessary to study the optimal proportion of microencapsulation of kaffir lime peel essential oil to produce microencapsulations that have efficacy as anticellulitis caused by *Staphylococcus aureus.*

Materials and Methods

Materials

A set of distillation tools, a dispersing device (Ultra-Turrax) (IKA T 18 basic), glassware, tissue, pipettes, test tubes, ose needles, petri dishes, PSA instruments (Particle Size Analyzer), magnetic stirrer (HMS-79), freeze dryer (TOPT-10B), whatman filter paper, GC-MS instrument (Gas Chomatography-Mass Spectrometry), bacterial incubator, and SEM instrument (Scanning Electron-Microscopy) (Phenom TM).

The materials used in this study include: kaffir lime peel, aquadest, anhydrous sodium sulfate (Na_2SO_4), amoxicillin, maltodextrin powder ($C_6nH(10_n+2)O(5n+1)$) technical, chitosan powder (C_6HnO_4)n, n-hexane (C_6H_{14}) pa, Tween 80, nutrient agar, Mueller-Hinton, nutrient broth and culture *Staphylococcus aureus*.

Kaffir Lime Peel Oil Isolation

The isolation process of kaffir lime peel essential oil was carried out by weighing 10 kg of kaffir lime fruit then peeling the kaffir lime peel and collecting it in a container. The kaffir lime peel is mashed using a blender. Kaffir lime peel (fine) and put 10 L of well water into a water distillation kettle. The water distillation process was carried out for 4 hours. The oil is accommodated in a beaker glass and then added Na_2SO_4 anhydrous. Furthermore, the physical test (smell, color), refractive index test using a refractometer, optical rotation using a polarimeter, and density. Obtained kaffir lime peel essential oil.

Microenkapsulation Process

6 grams of encapsulant was made from the composition of chitosan: maltodextrin in the ratio (0.1:5.9 ; 0.3:5.7 ; and 0.5:5.5). Then 100 ml of distilled water was added and mixed evenly by homogenization at a rotation speed of 6000 rpm at a temperature of 50°C for 15 minutes. Furthermore, 10 ml of kaffir lime peel essential oil was added to the encapsulation material and homogenized using a dispersing machine (Ultra-Turrax) at a speed of 15,000 rpm for 15 minutes and addedtween 80 drop by drop while stirring. The microcapsules produced at this stage were then stored in a petri dish and put into a cup freezer until frozen for process freeze drying. The frozen microcapsules were obtained.

Freeze Drying Process

Drying process using freeze drying with a temperature of less than -47 °C. Then a solution of encapsulation of kaffir lime essential oil was prepared in a frozen petri dish and then mounted on the instrument freeze dryer. Microencapsulated powder was obtained.

Characterization of Microencapsulation

The characterization test process is carried out by entering the essential oil into the instrument Gas Chromatography (GC-MS) to determine the active compound in the essential oil of kaffir lime peel and inject the microencapsulated into the instrument Gas Chromatography (GC) to determine the active compound of the microencapsulated ,Particle Size Analyzer (PSA) to determine the particle size of the microencapsulated, and Scanning Electron Microscope (SEM) to determine the surface morphology of the microencapsulated.

Bacterial Diffusion Test

A solid medium was prepared in a petri dish for each variation of the microencapsulated, in each petri dish the kaffir lime peel encapsulate was added as a test result, antibiotics amoxicillin as a positive control, and aquadest as a negative control. Each petri dish was put into the incubator for 1x24 hours, 2x24 hours, and 3x24 hours. Bacterial activity was found *Staphylococcus aureus*.

Results and Discussions

Kaffir Lime Peel Oil Isolation

In the process of isolating kaffir lime, first peeling the kaffir lime peel and blending. The purpose of the blender process is to increase the surface area of the sample which will facilitate the hydrodiffusion process, namely the process of penetrating steam into plant tissue and taking oil from plant tissue with the help of hot steam formed in the distillation process. Then weighed and obtained kaffir lime peel as much as 4.16 kg. Then the water distillation process is carried out. Water distillation has the principle that the material to be distilled is connected directly to boiling water or in other words, boils plants directly. In this study using water distillation because the sample used has a solid and hard form, the sample will be in direct contact with perfectly soaked water. In the water distillation process, hot steam will penetrate the plant tissue and be transported, then the steam will leads to the straight connector to the condenser and is converted into a liquid or liquid phase (condensate). The distillation process was carried out for 3 hours and the condensate liquid (oil and hydrosol) were separated using a separator, then the kaffir lime peel hydrosol was collected in a basin and the kaffir lime peel essential oil was

accommodated in a 250 mL beaker glass. Kaffir lime peel essential oil was obtained as much as 153.6 mL. Then the kaffir lime peel essential oil is added with Na_2SO_4 sufficient anhydrous. This addition serves to remove the remaining hydrosol or water that is still present in the essential oil.

Kaffir Lime Peel Essential Oil Analysis

The analysis of kaffir lime peel essential oil was carried out by determining the physical properties of the oil in the form of appearance, color, odor, refractive index, density, and optical rotation of the kaffir lime peel essential oil. Comparison of research results with Standard Essential Oil Association as of sweet orange peel in ISO 3140:2011. The straight comparison could not be found beacuse there is no such standard for kaffir lime peel oil. Table 1 showed the descriptions of the each parameter.

Table 1. Comparison of physico-chemical test of kaffir lime peel essential oil with ISO 3140:2011

No	Parameter	Comparison	
		Sweet orange peel (ISO 3140:2011)	Kaffir lime peel in this study
1	Color	Light yellow – Colorless	Colorless
2	Smell	Typical kaffir lime	Typical kaffir lime
3	Density	0.8494 - 0.8653	0.8533
4	Refractive Index	1,471 - 1,475	1.466
5	Optical rotation	(+)16.95	(+)13.20
6	Rendement	Minimum 1.42 %	3.12%

From the results of the analysis of the kaffir lime peel essential oil as shown in Table 2, it is obtained The mass of kaffir lime peel oil is 129.87 grams. The test for the essential oil content of kaffir lime peel was carried out by Gas Chromatography Mass Spectrometry (GC-MS) to determine the percentage and amount of compounds contained in the essential oil of kaffir lime peel. The chromatogram of the separation of the components of the kaffir lime peel oil is shown in Figure 1

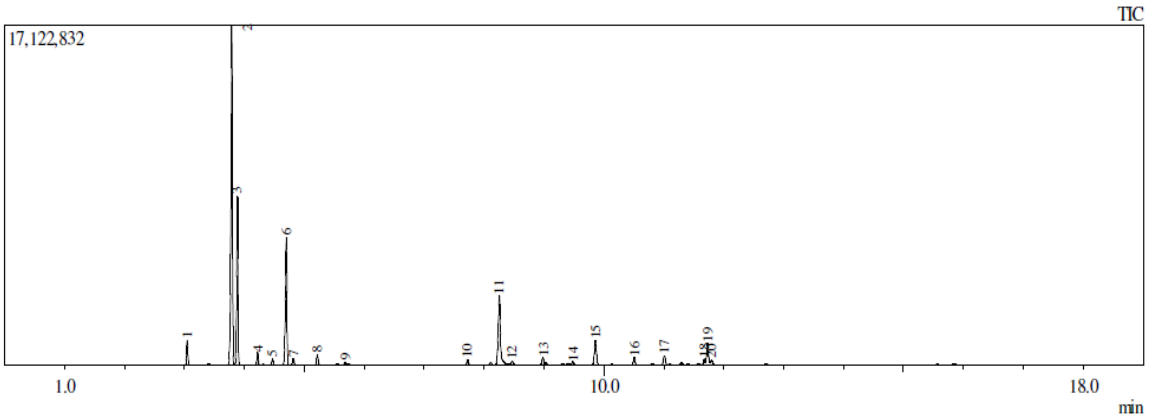

Figure 1. Chromatogram of kaffir lime peel essential oil

From the results of the GC-MS analysis as shown in Figure 1, the main compound, namely kaffir lime peel essential oil, contains the main components, β-pinene (peak 2) 35.89%, limonene (peak 3) 14.95%, citronellal (peak 6)) 12.31%, and terpinen-4-ol (peak 11) 3.91%. The results of the essential oil identification test using GC-MS stated that the kaffir lime peel from Pasar Pakem, Sleman contained 20 chemical compounds with 5 main components. The chromatogram in Figure 1 contains the active ingredient compounds from kaffir lime peel essential oil as shown in Table 2

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Table 2. Active compounds from kaffir lime peel essential oil

Peak	Ret. Time (min)	Compounds	Area
2	3,789	*beta pinene*	26584500
3	3,882	*limonene*	12571632
6	4,700	*sitronellal*	11071477
11	8,253	*terpinen-4-ol*	9118007

Microencapsulation of Kaffir Lime Peel Essential Oil

Encapsulation was part of material technology to gain more shophisticated, advanced and flexible utilities of certain purpose and application. Micro or nano encapsulation were based on how the selected matrix and core/active ingredients could be homogenized in the process.In this study, variations in the ratio of chitosan: maltodextrin coating were used of 0.1: 5.9, 0.3: 5.7 and 0.5: 5.5 grams. The microencapsulant is inserted into thefreezer until frozen for process freeze drying. A frozen microcapsule was obtained as shown in Figure 2.

Figure 2. Chitosan product on microencapsulated powder: Maltodextrin (0.1 :5.9) grams (A), (0.3:5.7) grams (B), (0.5:5.5) grams (C).

The obtained powder has a different texture and mass as shown in Table 3

Table 3. Efficiency of microencapsulation

Mass Ratio (Chitosan : Maltodextrin) (w/w)	Mass Before drying (g)	Mass After drying (g)	Efficiency (%w/w)
A (0.01 : 1)	5	3.47	69.4
B (0.05 : 1)	5	2.89	57.8
C (0.09: 1)	5	1.52	30.4

In Table 3, the coating variation shows the results of the microencapsulated in perfect powder form. In this study, it can be concluded that there is a difference in powder form due to variations in the chitosan coating, where the less chitosan, the more perfect powder form.

Figure 2 shows the results of the microencapsulated which has been dried in a freeze dryer. Figure (A) is a microencapsulate with a variation of 0.1 gram chitosan coating: 5.9 gram maltodextrin, Figure (B) is a microencapsulate with a variation of 0.3 gram chitosan coating: 5.7 gram maltodextrin and Figure (C) is a microencapsulate with a variation of 0.5 grams of chitosan coating: 5.5 grams of maltodextrin. In Figure (A) shows the results of the microencapsulate in the form of a perfect powder, in Figure (B) shows the results of the microecapsulate in the form of a powder but not uniform and in Figure (C) shows the

results of the microecapsulate in an irregular round shape but not in a perfect powder form. In Figure 2 it can be concluded that there is a difference in powder form due to variations in the chitosan coating, where the less chitosan the more perfect powder form. The physical microencapsulation efficiency is obtained by the formula:

$$\text{Efficiency} = (\text{Weight after drying})/(\text{Weight before drying}) \times 100\%$$

The physical efficiency of microencapsulation in variation A is 69.4%, variation B is 57.8%, and variation C is 30.4%. It can be concluded that variation A gets the best physical efficiency with an efficiency of 69.4%. The reduction in weight before and after drying occurs because during the drying process in the freeze dryer there is quite a lot of water expenditure. In this study, it was concluded that the more the amount of chitosan added, the lower the efficiency, namely by 30.4% and the more the amount of maltodextrin added, the higher the physical efficiency, which was 69.4%.

Analysis of active compounds using Gas Chromatography

The resulting microencapsulates were analyzed for the content of the active ingredients using an instrument Gas Chromatography to produce the best variation. The best variation was obtained, namely variation A with a ratio of 0.01: 1, with a chromatogram as shown in Figure 3.

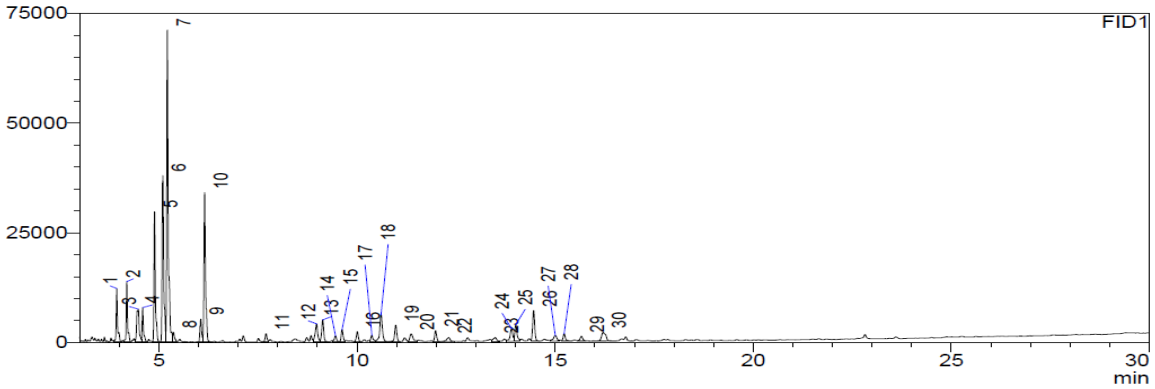

Figure 3. Microencapsulated chromatogram after freeze drying

The chromatogram in Figure 3 still contains the active ingredient compounds from kaffir lime peel essential oil as shown in Table 4.

Table 4. Comparison of Active Compound

Peak	Ret. Time (min)	Compounds	Area
6	5,099	*beta pinene*	98550
7	5,215	*limonene*	189800
18	10,608	*sitronellal*	28544
19	10,977	*terpinen-4-ol*	15622

Based on Table 3, it can be concluded that the microencapsulation results were successful in coating the active ingredient content of the kaffir lime peel essential oil.

Particle size analysis with Particle Size Analyzer (PSA)

Microencapsulates were analyzed for particle size using the instrument Particle Size Analyzer (PSA), the results are shown in Figure 4.

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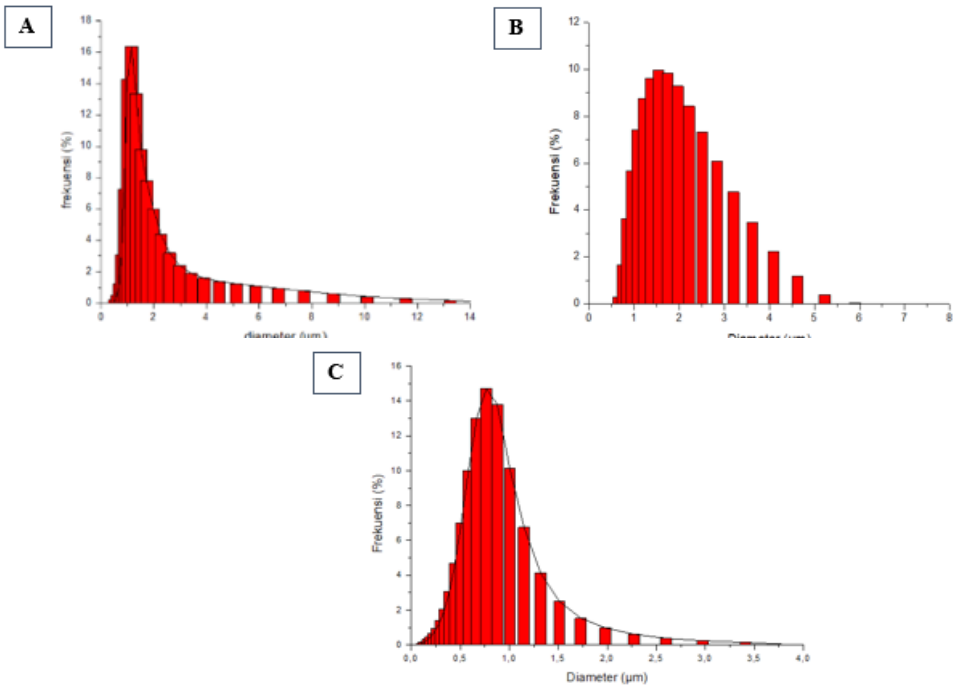

Figure 4. Particle Size Distribution

Based on the measurement results as shown in Figure 4, the particle size ranges from 0.510 m to 13,428 m with an average particle size of microencapsulated kaffir lime peel essential oil of 1.72 m.

Microencapsulated morphology analysis with Scanning Electron Microscope (SEM)

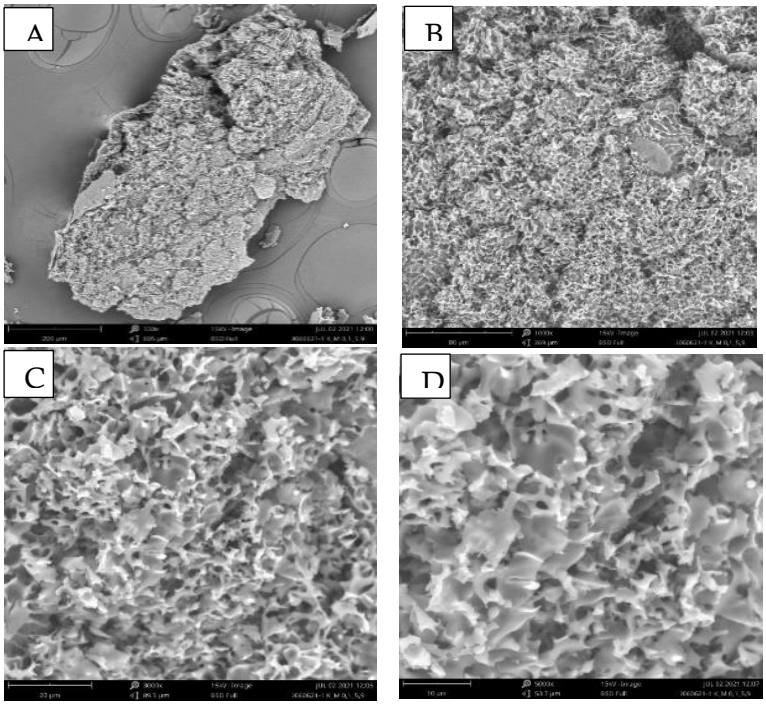

Figure 5. Microencapsulated Morphological Structure (A) magnification 330 times, (B) 1000 times magnification, (C) 3000 times magnification and (D) 5000 times magnification

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The resulting microencapsulates were analyzed using Scanning Electron microscope (SEM) to determine the surface morphology of the microencapsulated. The morphological structure of the microencapsulated at a magnification of 330 times shows the shape of the microencapsulate, which is round and oval with no cavities in the microencapsulate. At 1000 times the magnification of the microencapsulated, the surface morphology of the microencapsulated was irregular. At 3000 times magnification, the microencapsulates appear as white, irregular crystals. At a magnification of 5000 times it is clearer that the microencapsulated structure does not have pores or cavities. The results of the SEM analysis prove that the oil contained in the microencapsulate is evenly distributed.

Antibacterial activity test by diffusion method

In this study, the antibacterial activity was tested using diffusion method and the clear zone was measured as shown in Figure 6.

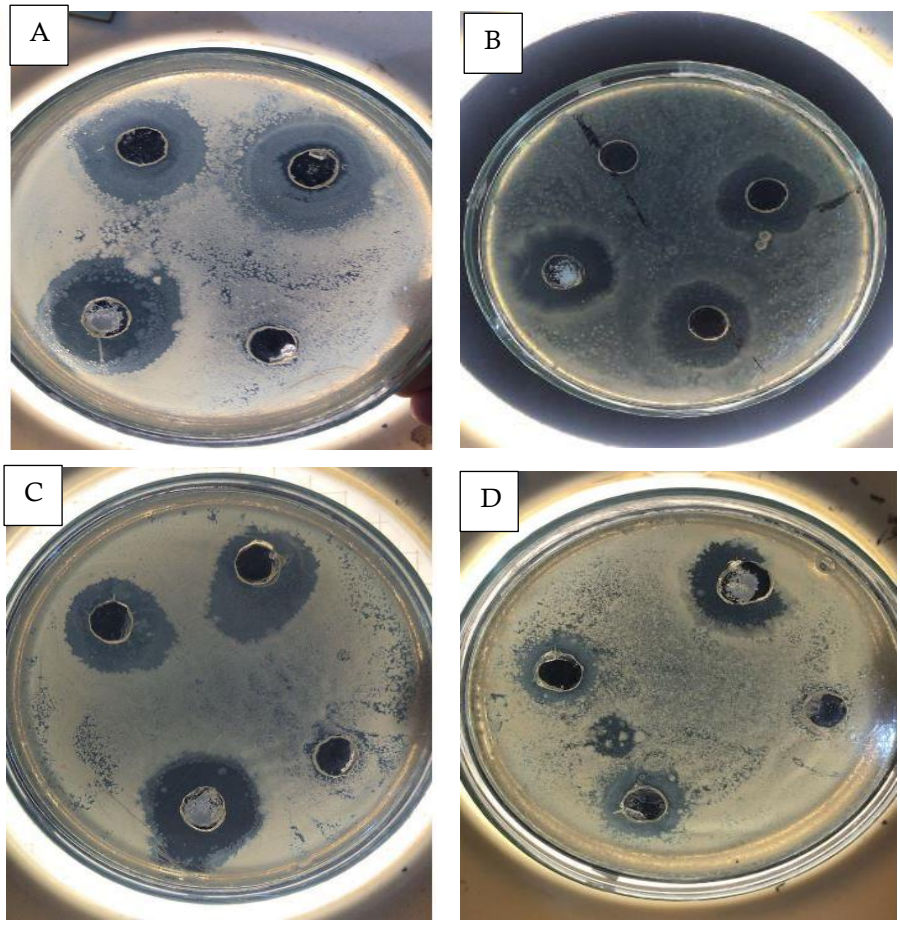

Figure 6. Observation of the 3rd day of bacteria *Staphylococcus aureus* variation of chitosan 0.1 grams (A), 0.3 grams (B), 0.5 grams (C), and Essential oil extract without microencapsulation

Based on Figure 6, the diameter of the bacterial inhibition zone is obtained *Staphylococcus aureus* as in Table 5

Table 5. Clear Zone Of Bacteria *Staphylococcus aureus*

Observation	Time			
	1x 24 hours	2 x 24 hours	3 x 24 hours	Inhibitory Activity
Antibiotics	22 mm	24 mm	24,5 mm	Very Strong
A (0.1 gram chitosan : 5.9 gram Maltodextrin)	21 mm	22 mm	22,5 mm	Very Strong
B (0.3 gram chitosan : 5.7 gram Maltodextrin)	20 mm	19,5 mm	20 mm	Strong
C (0.5 gram chitosan : 5.5 gram Maltodextrin)	20,5 mm	21 mm	20,5 mm	Very Strong
D Essential oil extract without microencapsulation	7 mm	6 mm	5,5 mm	Weak

Based on the test results of antibacterial activity *Staphylococcus aureus* as in Table 5 on days 1-3 the best clear zone is formed, namely in variation A 0.1 grams of chitosan and 5.9 grams of maltodextrin with a clear zone on the last day of observation of 22,5 mm. Based on these results, it can be concluded that the inhibitory activity of kaffir lime peel microencapsulates has a very strong inhibitory activity so that kaffir lime peel microencapsulates can kill bacteria *Staphylococcus aureus* cause of cellulitis. Then the results of the antibacterial activity test microencapsulated compared with extract of kaffir lime peel essential oil without microencapsulation process Based on the test results of antibacterial activity *Staphylococcus aureus* as in Figure 6 (D) shows the results of essential oil extracts without the microencapsulation process against bacteria Staphylococcus aureus as a cause of cellulitis is not effective, because the clear zone produced is not large which indicates that the inhibition against bacteria is weak, so it can be concluded that the microencapsulated product is much better than the kaffir lime peel essential oil extract product without the microencapsulation process.

Conclusion

Based on the test results of antibacterial activity *Staphylococcus aureus* as the cause of cellulitis, namely on day 1-3 the best clear zone is formed. namely in variation A, namely 0.1 grams of chitosan and 5.9 grams of maltodextrin with a clear zone on the last day of observation of 22.5 mm. Based on the results of antibacterial activity testing, microencapsulation of kaffir lime peel essential oil has a very strong inhibitory effect and has bacteria-killing properties.Staphylococcus aureus so it is effective to be an anticellulitis powder preparation

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