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PREPARATION OF KEMUNING LEAF EXTRACT MICROENCAPSULATION BY IONIC GELATION METHOD AS AN ANTIBACTERIAL

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Abstract. Extracts from the leaves of the kemuning plant have bioactive compounds that have potential as antibacterial. However, there are some difficulties when using bioactive compounds such as instability, reactivity and having a strong odor. To overcome this problem, it is important to carry out the treatment, namely, the microencapsulation technique. Microencapsulation is a technique for coating the active ingredient with a polymer that can protect the active component thereby increasing the bioavailability of the active compound to be covered by the coating material. The polymer materials used as coating materials in this study were chitosan and STTP using the ionic gelation method. The characteristics measured included the phytochemical test, PSA test, antibacterial, FTIR, and SEM. Phytochemical test results showed the presence of alkaloids, flavonoids, PSA test showed micro-sized encapsulation, antibacterial indicated the presence of a strong inhibitory zone which had potential as antibacterial, FTIR indicated the presence of OH, C=O, NH groups.

Keywords: Microencapsulation, Antibacterial, Kemuning Leaf Extract, Ionic Gelation.

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1 INTRODUCTION

The kemuning plant (*Murraya paniculata* (*L.*) Jack) is a small tropical evergreen shrub, native to tropical and subtropical parts of the world that belongs to the Rutaceae family, Genus Murraya, Species *Murraya paniculata* (*L.*) Jack. Murraya paniculata (L.) Jack is commonly used in traditional medicine for the treatment of diarrhea, colic, stomach pain, dysentery, headache,

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edema, thrombosis and blood stasis. Extracts from bark and leaves have potential as anti-inflammatory, antidiarrheal, antidiabetic, antimalarial, antibacterial, antifungal, and antioxidant activities.[1], [2], [3].

Murraya paniculata (L.) Jack is rich in various active substance components. The most frequently found substances are alkaloids, flavonoids and coumarins. In addition, *Murraya paniculata* (*L.*) Jack also contains 60 compounds identified from the extraction of the leaves, including γ-elemene, perolidol, tcaryophyllene, caryophyllene oxide, βcaryophyllene, spathulenol, β-elemene, germacrene D and cyclooctene, 4-methylene (propenylidene) and many more[4].[5] has conducted research on kemuning leaf extract. Where the results of the study showed the presence of active compounds such as alkaloids, flavonoids contained in kemuning leaf extract which have the potential as antipseudomonas. [6] also conducted research on the antibacterial test of kemuning leaf extract against *Staphylococcus aureus* and *Escherichia coli* bacteria showing the results of an inhibition zone for these bacteria. In this study, the focus will be on testing the antibacterial activity found in the active ingredients of the Kemuning leaf extract. Antibacterial potential in kemuning leaves is influenced by the presence of active compounds of flavonoids. However, there are some difficulties when using bioactive compounds such as instability, reactivity and having a strong odor. To overcome this problem, it is important to do treatment. The treatment currently being developed is the microencapsulation technique.

Microencapsulation is emerging as a potential approach to overcome this problem and at the same time to provide controlled or targeted delivery or release [7]. Microencapsulation has been applied in a wide variety of products and research has shown that the use of encapsulation techniques can produce superior quality products [8].

Microencapsulation is a technique for coating the active ingredient with a polymer that can protect the active component thereby increasing the bioavailability of the active compound to be covered by the coating material. The polymer material used as a coating material in this study is chitosan. Chitosan was chosen as a coating material because of its general use in microencapsulation techniques, besides that chitosan is non-toxic, biodegradable and biocompatible. In the manufacture of microencapsulation, it can be done by several methods, one of which is the ionic gelation method. Ionic gelation is the main technique for ionic interactions using chitosan as a polycationic compound. In the ionic gelation technique, microencapsulation is also formed by a polyanion compound such as sodium tripolyphosphate. This method is one of the most commonly used methods, using chitosan as a coating agent and sodium tripolyphosphate as a crosslinking agent [9]. The main advantage of this method is that it is easy to perform, without the use of hazardous organic solvents, without heat or strong agitation [10]. [11] have successfully encapsulated herbal galactagogue extract in CS-TPP microcapsules using ionic gelation method with mean diameter of 27 μm and EE% of 83.054%. FTIR analysis revealed successful extract loading in alginate microcapsules. Based on the above background, a study was conducted on the

microencapsulation of kemuning leaf extract using the ionic gelation method which has antibacterial potential.

2. METHODOLOGY

2.1 Preparation of Kemuning Leaf Extract

1000 g of kemuning leaf washed and cleaned, then heated in the oven at 40°C for 24 hours. After that, grind it using a blender and filter the kemuning leaf powder. After that, the kemuning leaf powder was put into a plastic bottle and added with 70% ethanol solvent with a ratio of 1:10. Soaking time was 24 hours at room temperature and stirring was carried out. The filtrate is then filtered using filter paper, then the solvent is evaporated using a rotary evaporator at a temperature of 40-50°C, then concentrated over a water bath until a thick extract is obtained. Furthermore, the kemuning leaf extract will be made in the form of microencapsulation.

2.2 Preparation of Kemuning Leaf Extract Microencapsulation Suspension

2.2.1 Preparation of STPP Solution

500 mg of Sodium Tripolyphosphate (STTP) was dissolved in 50 mL of distilled water to produce STPP solution and then homogenized using a magnetic stirrer at 1500 rpm for one hour.

2.2.2 Making Chitosan Solution

50 mg of chitosan powder was dissolved in 50 mL of 1% glacial acetic acid solution and then homogenized using a magnetic stirrer at 1500 rpm for one hour to produce a chitosan solution.

2.2.3 Preparation of Kemuning Leaf Extract Microencapsulation Suspension

Dropped slowly 5 mL of kemuning leaf extract with 50 mL of chitosan solution and stirred for 30 minutes. Then it was dripped slowly with 50 mL of STPP with a solution ratio of chitosan: STPP, namely 1: 1, stirred for 30 minutes and ultrasonicated for 10 minutes. Furthermore, the microcapsule suspension was subjected to morphological characterization of FT-IR, PSA, and antibacterial test.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Test Results

The Phytochemical Screening Test was carried out to qualitatively determine the presence or absence of bioactive compounds contained in the kemuning leaf extract. The results of the kemuning leaf extract screening test can be seen in the following table.

Table 1. Results of the Phytochemical Screening of Kemuning Leaf Extract.

Phytochemical Test	Positive Results According to Readers	Results
Alkaloids	A brick red precipitate formed (Dragendrof reagent)	+

	A yellowish white precipitate (Mayer's reagent) is formed.	+
Flavonoids	The presence of black colloid	++
Saponins	There is foam that lasts ± 10 minutes	+
Steroids, Terpenoids	Brownish or violet ring	-
Tannins	Formed dark blue or greenish black	+

Information :

++ : contains more/more concentrated compounds

+ : contains less compound

- : does not contain compounds

From the results of the phytochemical screening test, it was found that there were alkaloids, flavonoids, saponins and tannins in the kemuning leaf extract.Several studies have found that kemuning leaf extract contains antibacterial properties due to the presence of metabolites [5]. [12] states that kemuning leaf contain chemical compounds which are secondary metabolites such as saponins, alkaloids, tannins, and flavonoids. Due to the presence of secondary metabolites in the kemuning leaf extract, the secondary metabolites are richpenetration into the cell so that it can disrupt the peptidoglycan component in the bacterial cell, causing the cell wall to not form properly and the cell itself to die.

3.2 Particle Size Analyzer

Particle size is analyzed using a particle size analyzer called a particle size analyzer (PSA). PSA results show the mean1.00132 (µm), where the particle size category is in micro sizewith diameters ranging from 1 µm to several 100 µm [13]. The results of the particle size analysis can be seen in the following figure.

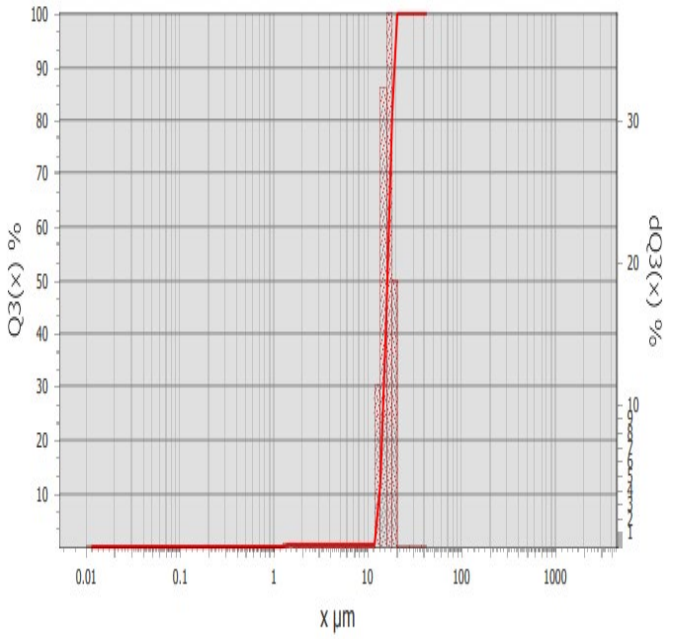

Figure 1 Graph of Microencapsulated Particle Size of Kemuning Leaf Extract

3.3 Fourier Transform Infrared Spectroscopy

The results of the FTIR test on microencapsulation were carried out to see whether the encapsulation process was going well by looking at changes in wave numbers resulting from mixing chitosan, STTP, and kemuning leaf extract. From the picture above it can be seen that the encapsulation process is going well. Where there is a change in wave number when mixing chitosan, STTP, and kemuning leaf extract. The FTIR results can be seen in the following figure.

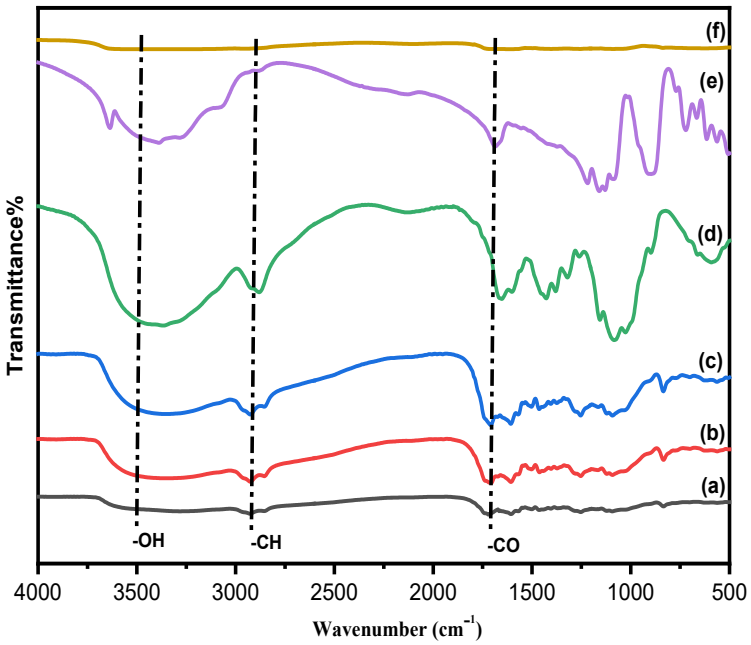

Figure 2 FT IR spectra of 10 mL kemuning leaf extract microencapsulation (a), 7.5 mL kemuning leaf extract microencapsulation (b), 5 mL kemuning leaf extract microencapsulation (c), chitosan (d), STTP (e), leaf extract kemuing (f)

Based on the picture above, it can be seen that there are absorption bands in the microencapsulation of 10, 7.5, 5 ml of kemuing leaf extract at a wave number of around 3468 cm-1 which indicates the OH and NH stretching vibrations involved in hydrogen bonding where the kemuning leaf extract is added, the more strong stretch. This is influenced by the clusters contained in the kemuning leaf extract which are added the greater. In the 1730 cm-1 group there is a C=O group due to the groups found in chitosan. At 1256 cm-1 indicates an asymmetric stretching vibration of the PO2 group that connects the tripoliphosphate ion, this indicates that the tripoliphosphate anion group is bound to the protonated amino group of chitosan which proves a cross-link is formed [14].

3.4 Antibacterial Test Results

Antibacterial test was conducted to determine the potential of microencapsulation of kemuning leaf extract in inhibiting bacterial growth. The antibacterial results can be seen in the

following figure.

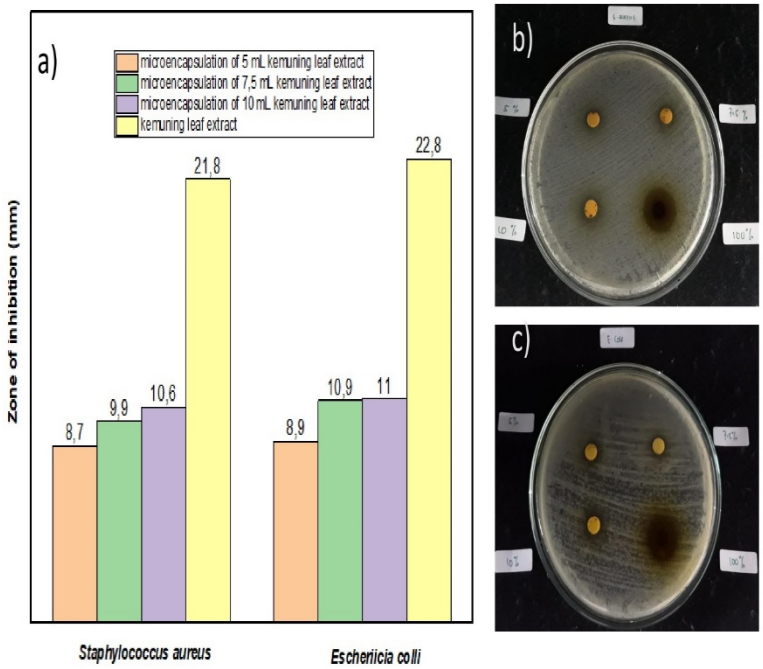

Figure 3 diagram of the antibacterial inhibition zone (a), *Staphylococcus aureus* inhibition zone diagram (b), *Eschericia colli* inhibition zone diagram (c)

Testing the antibacterial activity of microencapsulation of kemuning leaf extract showed the formation of a clear zone around the disc soaked with kemuning leaf extract, microencapsulation of 5, 7.5, and 10 mL of kemuning leaf extract. The largest inhibition zone was found in the kemuning leaf extract, which was 21.8 mm in bacteria *Staphylococcus aureus* and 22.8 mm in *Escherecia colli* bacteria. This is influenced by the absence of a mixture of chitosan solution and STTP as a coating material to protect the active ingredients from the kemuning leaf extract. The inhibition zone on the microencapsulation of the kemuning leaf extract on 5 mL of the kemuning leaf extract was 8.7 mm and 8.9 mm, on the microencapsulation of the kemuning leaf extract 7.5 mL was 9.9 mm and 10.9 mm, on the microencapsulated kemuning leaf extract 10 mL of 10.6 mm and 11 mm in Staphylococcus aureus and Escherecia colli. The inhibition zone on microencapsulation is the largest on microencapsulation containing 10 mL of kemuning leaf extract was still in the strong category of inhibition zone. Inhibition according to Davis and Stout (1971) is divided into: very strong (inhibition zone> 20 mm), strong (inhibition zone 10-20 mm), moderate (inhibition zone 5-10 mm), and weak (inhibition zone <5 mm) [15]. Therefore, microencapsulation of 10 mL of kemuning leaf extract is a microencapsulation that has the potential as a strong antibacterial material, which is good for use as an antibacterial material.

4. CONCLUSION

Based on the results of the research that has been done, it can be concluded that the preparation of microencapsulation of yellow leaf kemuning using the ionic gelation method has been successfully encapsulated in the form of micro particle sizes. The formation of encapsulation can be seen from the presence of OH, NH, CO functional groups in the microencapsulation. The

addition of the volume of the kemuning leaf extract can increase the strength of the inhibition zone of the microencapsulation, the highest inhibition zone is found in the 10 mL microencapsulation of the kemuning leaf extract.

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