Effectiveness of Maltodextrin as a Coating in Microencapsulation of Clove Leaves Oil (Syzygium aromaticum) for Antibacterial Applications

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Abstract. Clove leaves oil is potential oil used as antibacterial such as Escherichia coli. However, clove leaves oil has several disadvantages, including being easily oxidized and volatile. In order to overcome this problem a microencapsulation process is carried out. The purpose of this study was to test the ability of clove leaves oil and the results of microencapsulation as an antibacterial E. coli. Clove leaves oil was isolated using water-steam distillation. Furthermore, the obtained essential oil was microcapsulated by spray-drying method. Microencapsulation results were characterized using SEM (Scanning Electron Microscope) to determine the morphology of the produced material. The SEM results indicate that the resulting microcapsules are rounded shape, which shows that the formed microcapsules have not fully developed. The results of the activity test by using clove leaves oil as an antibacterial E. coli denoted a inhibitory zone diameter was 15.2 mm, while the oil was microencapsulated with a coating ratio of 1:4, 1:6, 1:8, 1:10, 1:12 produces inhibitory zones 9.9; 11.3; 12.9; 14.8 and 17.5 mm, respectively. Results of statistical tests indicate that antibacterial activity of clove leaves oil is 100 % significantly different from the comparative microencapsulation activity 1:4 with t_value = 11.30>t_tab = 9.93. Ratio microcapsul 1:6; 1:8; 1:10 and 1:12 having activities that were no different from clove leaves oil 100% and were showed by t_value 8.65; 4.25; 0.76; 2.62, respectively < t_tab = 9.93 on the confidence level α = 0,01; df =2. Thus it can be stated that minimum ratio of maltodextrin to clove leaves oil that can be used as a coating is a microcapsule ratio of 1: 6.

1. Introduction

Clove (Syzygium aromaticum) is one of essential plants that have been widely cultivated in Indonesia. In the trade that is commonly sold from cloves is clove oil. Clove leaves oil contains many chemical compounds, the most important of which is composition of eugenol [1]. Eugenol compounds are main components contained in clove oil with a content up to 70-90%, thus clove oil contains several other components such as eugenol acetate and ß-caryophyllene [2]. Eugenol is a colorless or pale yellow liquid, which if necessary the sunlight turns it into dark brown with specific odor [1].

Clove oil has chemical properties and pharmacological effects that use anesthetics, antimicrobials, antiseptics, antioxidants, and immunomodulators [3]. The content of phenol compounds in this oil contains antibacterial, which can inhibit the growth of bacteria that work by poisoning the cytoplasm, damaging and penetrating the walls and depositing bacterial cell proteins. [4]

The clove leaves oil has several weaknesses including being easily oxidized and evaporated as
well as difficult to disperse in dried materials. One method that can be done to overcome this issue is by adopting microencapsulation. It is one of the most efficient methods to protect solids, liquids or even gases against the surrounding environment into microscopic particles protected by a wall material [5]. Microencapsulation can be used to protect fragrances or other active agents from oxidation caused by heat, light, moisture, from contact with other substances, in order to prevent evaporation of volatile compounds and control the release rate [6].

Based on the background, then further research on isolation of essential oil from clove leaves was conducted and followed by microencapsulation process with spray drying method. Through this process will obtain solid phase that allows people in using clove leaves as flavour. In addition, the antibacterial activity of *Escherichia coli* from microencapsulation was also tested.

2. Experimental Methods

2.1. Preparation and Treatment samples

Clove leaves were taken from the clove plantation in Pusuea Village, North Poleang District, Bombana Regency, Southeast Sulawesi. Then they were dried up for four days. The drying process was carried out at room temperature without direct sunlight. The dried clove leaves weighed 2.5 kg and then put into a steam-water distillation container, and the container was previously filled with as much as 28 liters of water. Steam-water distillation process was carried out for 3 hours. Distillates were placed in separating funnels that formed a layer of oil and a layer of water. The water layer was separated from the oil layer using a separating funnel, then 1% anhydrous MgSO₄ is removed any residual water [7].

2.2. Microencapsulation

The essential oil of distillation of water vapor was microencapsulated with a maltodextrin coating material having good oxidation resistance. The preparation of microemulsion was done using Tomazelli method [8]. The variations in composition between essential oil and maltodextrin are 1:4, 1:6, 1:8, 1:10, 1:12. O/W emulsion mixed with maltodextrin, and stirring at 4000 rpm for 3-5 minutes. The emulsion was fed into spray-dryer (merk LabPlant type SD 05) at room temperature with a flow rate 300 mL min⁻¹. The inlet and outlet temperatures were maintained at 110°C and 68°C, respectively.

2.3. Surface Morphology Analysis with SEM

The test was performed by placing the sample in a carbon-conductive layer and coated with 60% gold and 40% palladium with sputter coater at a current of 35 mA for 1 min. Operating conditions were carried out at a voltage acceleration of 10 kV and 250x, 1000x, 5000x and 10000x magnifications [9].

2.4. Culture of Test Microorganisms

The nutrient agar medium (2% pepton, 1.5% yeast extract, 4% agar, and 1% NaCl). This medium was prepared by dissolving 22.1 g of nutrient agar with 260 ml of aquadest in erlenmeyer. The NA medium was sterilized in an autoclave at 121°C for 15 minutes [10]. The microorganism used *Escherichia coli* ATCC 25923. The bacteria was rejuvenated by transferring 1 or 2 ose from the stock of bacteria that has been supplied to the reaction tube containing 10 ml sterile liquid media (2% peptone, yeast extract 1.5%, NaCl 4%) and incubated for 24 hours at 37 ± 2°C [11].

2.5. Antibacterial Activity Testing

The NA medium was piped as much as 20 mL and then put into eppendorf tube and added with 10 µL inoculum of bacterium *E. coli* and then shaken until homogenous. After homogenous, it was poured into a petri dish with a circular motion until the media docked at a surface of petri dish, then let it stand for a few minutes until solid. It was then placed a disk paper (d:0.5 cm) that has been soaked in
the test solution of clove leaves oil (100%) and microcapsul of clove leaves oil (1:4; 1: 6; 1: 8; 1: 10 and 1:12), positive control of amoxicillin, negative control (tween oil and aquadest) on the surface of the solidified agar medium. After that, the petri dish was closed tightly and wrapped in plastic wrapping. It was then incubated for 1 x 24 hours in room temperature and the formed inhibitory zone was measured [12].

2.6. Data Processing and Analysis The Effectiveness of maltodextrin as a coating was determined using the t test by comparing antibacterial activity at each transition supported by the coating (1: 4; 1: 6; 1: 8; 1:10; 1:12) with antibacterial activity pure clove leaves oil (100%) with [13]:

\[
t_{\text{value}} = \frac{M_1 - M_2}{\sqrt{\frac{\sum_{i=1}^{N_1} S_i^2}{N_1 - 1} + \frac{\sum_{j=1}^{N_2} S_j^2}{N_2 - 1}}}
\]

t_{\text{value}} then compared with \( t_{\text{tab}} \) at a \( \alpha \) level of trust = 1%.

3. Results and Discussion

3.1. Clove Leaves Oil
Isolation of essential oil on fresh clove leaves in this study using the steam-water distillation method. During the refining process, water vapor will penetrate the tissue of clove leaves oil gland. Essential oil is removed through the hydrodiffusion process. The mixture of oil in water diffuses outward by osmosis, through a membrane that blooms to the surface of the material, and then evaporated by steam, which is flowed into the condenser. Distillation is collected into separating funnels, the oil layer and the water layer are separated for essential oil. Essential oil that still contain water molecules are dried up by adding anhydrous MgSO\(_4\). Anhydrous MgSO\(_4\) additional function for binding water contained in oil. In this study it produced a clear color with yield of 2.5%. Based on the test results using the GC-MS instrument in a previous study [14] obtained 2 main compositions of clove essential oil compiler consisting of 2 components namely eugenol (68.30%) and \( \beta \)-caryophyllene (27.43%).

3.2. Morphological Analysis of the Surface of Clove Leaves Oil Microcapsules with SEM
Clove leaves oil obtained was then microencapsulated and a white solid powder containing clove oil aroma was obtained. Figure 1. is a microencapsulated SEM clove leaves oil on the placement of clove leaves oil: maltodextrin: 1: 8. There is a small form of wrinkled ball on the appearance of the third SEM. Spherical particles represents that microcapsules are perfectly formed and contained clove leaves oil. When compared to Nurliana [7] research on microencapsulation of rogo leaves essential oil (Premna serratilolia L.) indicates a slight similarity forming microcapsules that are incompatible with those containing wrinkled balls. This causes the essential oil has not been fully filled in the coating in this case is mallodextrin.
3.3. Antibacterial Activity of Microcapsul of Clove Leaves Oil

The results and analysis of antibacterial activity of microcapsul of clove leaves oil inhibiting *E. coli* growth can be seen in Figures 2.

![Antibacterial Activity of Microcapsul of Clove Leaves Oil](image)

According to previously published studies, diameters of inhibitory zone were appreciated as follows: Insensitive (diameter ≤ 8.0 mm), moderate sensitive (8.0 < diameter < 14.0 mm), sensitive (14.0 < diameter < 20.0 mm), and extreme sensitive (diameter ≥ 20.0 mm) [15]. Figure 3. represents some differences in clove leaves oil microcapsules depending on moderate sensitive and sensitive categories.
Inhibitory zones were formed due to the presence of active substances in clove leaves containing antibacterial substances such as eugenol, which belongs to the phenylpropanoid group and β-caryophyllene, which belongs to the sesquiterpene group. These compounds can have toxic effects on bacteria. In addition, the hydrophobic nature of eugenol makes it easier to enter the lipopolysaccharide portion of the bacterial cell membrane, especially gram-negative bacteria and change the structure of the cell wall, so it causes intracellular leakage [16]. When phenol group compounds penetrate the bacterial cell membrane then interact with enzymes and proteins in the membrane it can cause adhesion of the bacterial cell membrane so that the osmotic pressure increases. This can cause damage to the cell membrane and inhibits bacterial respiration, which in turn causes the interference with the transfer of ions in the cell so that the bacteria died [17] [18].

The inhibitory zone is different for each variation required, different from the t test, in order to know whether it is significant or not.

**Table 1. t-Test results for various microcapsulation treatments on antibacterial activity of clove leaves oil**

| No. | Microencapsulation Oil: maltodextrin | Inhibitory zone 100% (X₂) (X2) | tₙₜ | tₜₜ (α = 0.01; db = 2) | Information |
|-----|-------------------------------------|---------------------------------|-----|-----------------------|-------------|
| 1   | 1:4                                 | 9.9                             | 11.30 | 9.92                 | Significantly different (100% > 1:4) |
| 2   | 1:6                                 | 11.3                            | 8.65  | 9.92                 | Insignificantly different           |
| 3   | 1:8                                 | 12.9                            | 4.25  | 9.92                 | Insignificantly different           |
| 4   | 1:10                                | 14.8                            | 0.76  | 9.92                 | Insignificantly different           |
| 5   | 1:12                                | 17.5                            | 2.62  | 9.92                 | Insignificantly different           |

Table 1 represents that only at the ratio 1: 4 is significantly different on antibacterial activity according to clove leaves oil activity at α = 0.01; db = 2. Meanwhile in other compositions, there is no significant difference between antibacterial activity of microencapsulation results using either 1:6; 1:8; 1:10; or 1:12 with antibacterial activity of clove leaves oil. This denotes that the use of maltodextrin as
a coating on composition determines the coating ≥ 1: 6 does not affect the activity antibacterial of E. coli.

4. Conclusion
The results of antibacterial activity test of clove leaves oil antibacterial and the results of microencapsulation are 100%, 1:4; 1:6; 1:8; 1:10 and 1:12 indicates different inhibitions 15.2; 9.90; 11.3; 12.9; 14.8 and 17.5 mm, respectively. While the t test results reported that antibacterial activity for clove leaves oil was 100% significantly different from microencapsulation activity results of 1: 4 involvement with $t_{\text{value}} = 11.30 > t_{\text{tab}} = 9.93$. Meanwhile microencapsulation involving 1: 6; 1: 8; 1:10 and 1:12 have activities that are no different from clove leaves oil 100% represented by $t_{\text{value}} = 8.65; 4.25; 0.76; 2.62$, respectively $< t_{\text{tab}} = 9.93$ on the confidence level $\alpha = 0.01; \text{df} = 2$. Thus it can be stated that minimum ratio of maltodextrin to clove leaves oil that can be used as a coating is a microcapsule ratio of 1: 6.

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