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MICROENCAPSULATION OF ESSENTIAL OIL FROM ROGO PLANT (*Premna serratifolia L.*) AS ANTIBACTERY *Escherichia coli*

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ABSTRACT

The microencapsulation of essential oil from rogo plant (*Premna serratifolia L.*) has been performed as an antibactery *Escherichia coli*. The purpose of this research is to know the component of composition, characteristic and test of essential oil activity of rogo and microencapsulation as antibactery E.coli. Rogo essential oil is obtained from isolation using steam-water distillation method with 0.63% rendamen. Characterization has been performed using Gas Chromatography-Mass Spectrometry (GC-MS) indicating that rogo isolated essential oil contains 4 main components such as eugenol (47.89%), eugenyl acetate (9.13%), massoil (29.78%) and cis-2-oxabicyclo, 4.4.0- decane (12.35%). The microencapsulation process of rogo essential oil is done using spray drying method with variation of rogo essential oil ratios: maltodextrin (1:10; 1:12; 1:14; 1:16; and 1:18) resulting in white sticky textured solid powder. The results of the rogo essential oil activity test showed that there was a decrease in concentration of 12.5%. While the result microencapsulated antibacterial activity test showed no difference from all variation concentration. The result of antibacterial activity test between rogo essential oil and microencapsulated results showed significant difference, the phase in the form of oil has greater antibacterial activity strength than the solid phase.

Keywords: *antibactery, microencapsulation, Premna serratifolia L.*

I. INTRODUCTION

Technological advances have an impact on lifestyle changes, including food consumption patterns. Communities consume more fast food, packaged and preserved foods. Food additives are widely used in everyday life. One of the synthetic additives commonly used as flavoring food is monosodium glutamate (MSG). MSG is the sodium salt of glutamic acid (Bera, et al., 2017)[1]. The use of MSG in excessive doses can lead to the disease of Chinese Food Syndrome or Chinese Restaurant Syndrome, neurotoxic effects on the brain, reproductive organ damage and obesity (Husarova and Ostatrivoka, 2013)[2].

Rogo plant (*Premna serratifolia* L.) is one such endemic plant in Celebes. Local people make use of rogo leaves as food additives that is as a remover of fishy smell in the meat as well as flavor because it has a fragrant aroma. In addition, they also take advantage of rogo plants as a drug fever and colds by boiling. Utilization of rogo leaves as flavoring and traditional medicine uses fresh leaves. However, fresh leaves have a short shelf life, higher microbial content and flavour consistency difficult to maintain. Essential oil as a flavoring giver is susceptible to high temperatures, oxidation, UV light, and moisture (Petrovic, *et al*, 2010)[3]. Microencapsulation is one of the most effecient methods to protect solids, liquids or even gases against the surrounding environment into microscopic particles protected by a wall material (Soliman, et al., 2013)[4]. Microencapsulation can be used to protect fragrances or other active agents from oxidation caused by heat, light, moisture, from contact with orther subtances over long shelf life, to prevent evaporation of volatile compounds and to control the release rate (Martins, et al., 2014)[5].

Based on the background, then further research on the isolation of essential oil from rogo leaves and followed the microencapsulation process with spray driying method. Through this process will be obtained solid phase that allows people in using rogo leaves as flavor. In addition, the antibacterial activity of *Escherichia coli* from the microencapsulation was also tested.

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II. MATERIALS AND METHODS

The equipment used in this research is a set of steam-water distillation apparatus, petromax stove, GC-MS (Gas Chromatography-Mass Spectrometry), Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) JSM-6510x, spray drying, incubator, Analytical balance (Acis), autoclave (Wisecclave), waterbath (HWS24), refrigerator (SHARP), micro pipette (DRAGON ONEMED), laminar air flow cabinet, shaker (Ratex), UV lamp, stative, clamp, hot plate, ruler, markers, eppendorf tube, ose needle, tip, petri dish, pyrex, measuring pipettes, ovens, dropper drops, vial bottles, dark bottles, knives, fresh rogo leaf samples (*Premnaserratifolia*L.), *E. coli*, maltodextrin, amoxilin, peptone 2%, agar 4%, 1% NaCl, tween oil, anhydrous $MgSO_4$, aquadest, kerosene, Whatman filter paper, gauze, sterile cotton, and aluminum foil.

Isolation of Essential Oils from Rogo Plant by Using Steam-Water Distillation

Fresh rogo leaves are weighed 2.5 kg and then put in a container of steam-water distillation device, the container was previously filled with water as much as 28 liters. The process of steam-water distillation is carried out for 3 hours. Destilates are accommodated in a separating funnel that forms a layer of oil and a water layer.The water layer is separated from the oil layer using a separating funnel, then added 1% anhydrous $MgSO_4$to remove residual water.

Component Analysis of Rogo Essential OilsCompound with GC-MS

Rogoessential oil is determined by the structure of the compound using GC-MS. The results obtained in the form of chromatogram and mass spectrum. The data obtained are further interpreted by comparing with the literature.

Microencapsulation

The volatile oil of distillation of water vapor is microencapsulated with a maltodextrin coating material having good oxidation resistance and can decrease viscosity. The preparation of microemulsionwasdone using Tomazelli method (2016)[6]. The variations in composition between essential oils and maltodextrin are 1:10, 1:12, 1:14, 1:16, 1:18.O/W emulsion mixed with maltodextrin, and stirring at 4000 rpm for 3-5 minutes. To obtain the microcapsules, the O/W emulsions were spray-dryer using a laboratory scale dryer (merkLabPlant type SD 05. The emultion was fed into spray-dryer at room temperature with a flow rate 300 mL min^{-1}. The inlet and outlet temperatures were maintained at 110^0C and 68^0C, respectively.

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 sputtercoater at a current of 35 mA for 1 min. Operating conditions were carried out at a voltage acceleration of 10 kV and 500x, 2000x and 5000x magnifications (Marcella, et al, 2015)[7].

Testing Rogo's Antibacterial Oil Activity

Sterilization of equipment and materials

All equipment is washed and dried. The vial bottle, test tube, erlenmeyer, petri dish wrapped in paper. Then it was sterilized by autoclaving at 121°C for 15 minutes. The aseptic work is carried out in a Water Label Laminar previously cleaned with a 70% alcohol solution, then sterilized with UV lamps lit for about 1 hour before being used in an antibacterial test (Sultana, *et al*., 2014)[8].

Media Manufacture and Sterilization

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 (Sultana, *et al*., 2014)[8].

Culture of Test Microorganisms

The microorganism to be used consist Escherichia coli ATCC 35219 bacteriaspesies. The bacteria are 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 (Sultana, *et al*., 2014)[8].

Antibacterial Activity Testing

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The NA medium was piped as much us 20 mL and then fed into efendorf tube and added 10 µL inoculum of bacterium Escherichia coli ATCC 35219 then shaken until homogenous. After homogenous poured in a petri dish with a circular motion until the media docked there is a surface of petri dish, then let stand a few minutes until solid. Then placed a disk paper (0.5 cm diameter) that has been soaked in the test solution (100%, 50%, 25%, 12.5%rogo oil and rogo oil: maltodextrin (1:10, 1:12, 1:14, 1:16, 1:18), positive control of amoxilin, negative control (tween oil and aquadest) on the surface of the agar medium that has been solidified). After that, the petri dish is closed tightly and wrapped in plastic wraping Then incubated 1 x 24 hours in room temperature Space and measured inhibit zone formed (Tomazeli, 2008)[6].

Processing and analysis of data

The data obtained separation data with GC-MS, SEM and zonal isolate values on antibacterial test.

III. RESULTS AND DISCUSSION

Isolation of Rogo Plant Essential Oils by Using Steam-Water Distillation

Isolation of volatile oil on fresh rogo leaves in this study used a method of steam-water distillation. During the distillation process, moisture will penetrate rogo leaf oil gland tissue. Essential oils are removed through the hydrodifusion process. This mixture of oil in the water diffuses outward with the event of osmosis, through the membrane that is blooming up to the surface of the material, and subsequently evaporated by the vapor passed to the condenser. The destilateis collected into a separating funel, oil layer and water layer were separated for essential oils. Essential oils that still contain water molecules are dried by adding anhydrous $MgSO_4$. Anhydrous $MgSO_4$addition function to bind the water still contained in the oil. In this study produced clear colored with rendamen of 0.63%.

Characterization of the Main Components of Rogo Plant Essential Oils

The result of GC-MS analysis rogoessential oil of obtained 4 components. Chromatogram essential oil of rogo is presented in **Figure 1.**

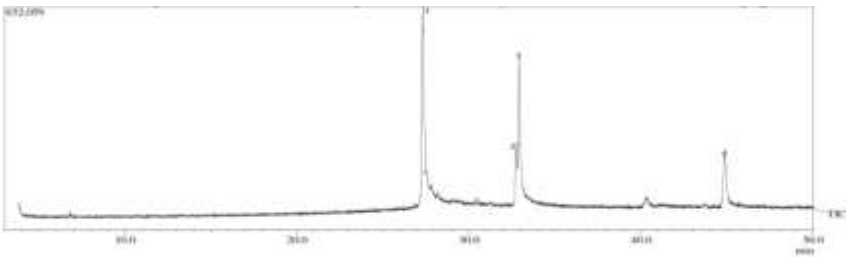

Figure 1. Chromatogram of rogo essential oil

The mass spectra of rogo essential oil component with a retention time of 27,308 minutes is presented in **Figure 2** below:

Figure 2. Mass spectra with Rt :27,308 minutes

Based on the mass spectrum it shows the presence of several peaks between, 164, 149 and 121 resulting from the termination of methyl radicals and CO molecules. Whereas the m/z: 91 and 65 peaks are the characteristic peaks of the aromatic compounds formed from the termination of β from aromatic rings to produce tropillary ions followed by the loss of acetylene molecules (Gross, 2011)[9]. These peaks are similar to the peak pattern of fragmentation of eugenol compounds in the WILLEY229 library can be seen in **Figure 3.**

ISSN: 2277-9655
Impact Factor: 5.164
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Figure 3. Standart mass spectra of eugenol compounds

The possible pattern of eugenol fragmentation is presented in **Figure 4** below:

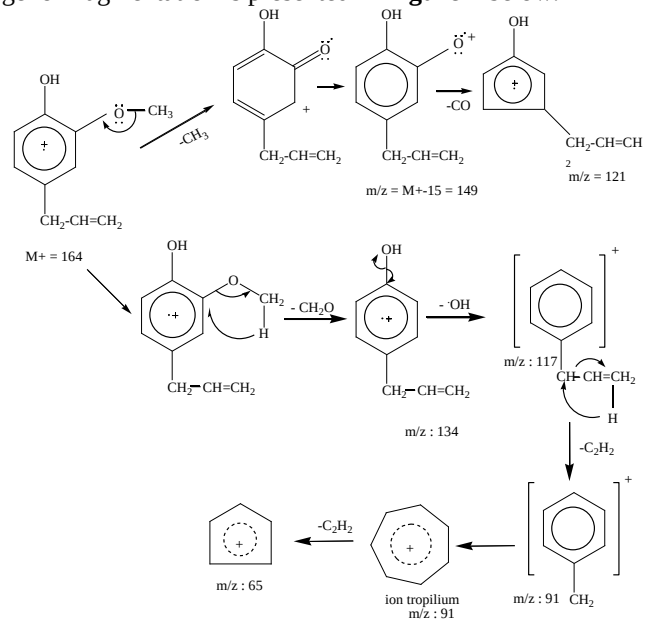


Figure 4. Hypothetical fragmentation pattern of eugenol

The mass spectrum of rogo essential oil component with a retention time of 32.742 minutes is presented in **Figure 5.**

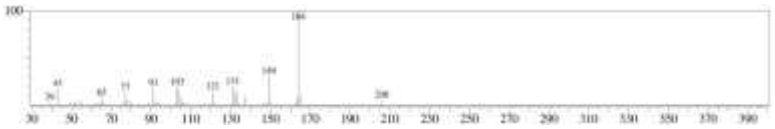


Figure 5. Mass spectrum with Rt :32.742 minutes

Based on the mass spectrum it Fig. 5shows the peak m/z: 206 which is the molecular weight of the compound,then there is a peak of 164 resulting from the breaking of CH_2CO molecules. Furthermore, as in eugenol compounds that produce peaks of 91 and 65. Peak 43 is formed from the breaking α of the ester group (Gross, 2011)[9]. These peaks are similar to the peak pattern of fragmentation of eugenyl acetate compound in WILLEY229 library can be seen in **Figure 6.**

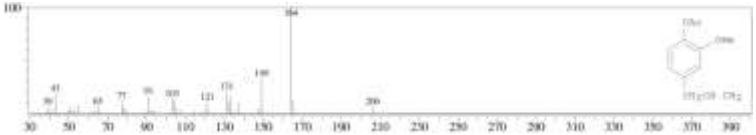
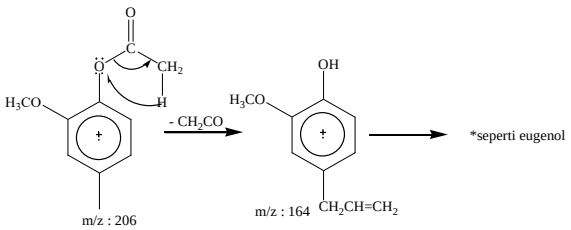


Figure 6. Standart mass spectra of eugenyl acetate compound

The possible pattern of eugenyl acetate fragmentation arepresented in **Figure 7**below:



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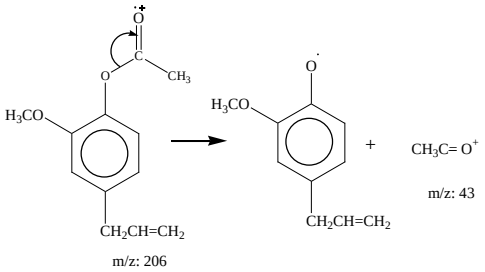


Figure 7. Hypothetical fragmentation pattern of eugenyl acetate

The mass spectra of the Rogo essential oil component with a retention time of 32.908 minutes are presented in **Figure 8.**

Figure 8. Mass spectra with Rt : 32.908 minutes

Based on the mass spectra it shows that the peak m/z: 97 and 68 is the result of the breaking α which is the characteristic disconnection of the ester compound (Gross, 2011)[9]. These peaks a similar to the peak pattern of fragmentation of the massoil compound in the WILLEY229 library shown in **Figure 9.**

Figure 9. Standart Mass Spectra of massoil compound

The possible pattern ofmassoil fragmentation are presented in **Figure 10.**

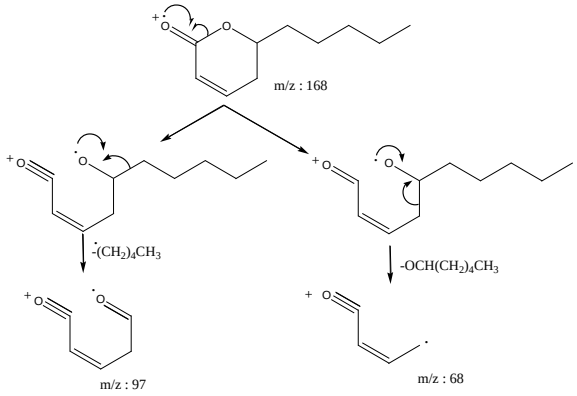


Figure 10. Hypothetical fragmentation pattern of massoil

The mass spectra of the rogo essential oil component with a retention time of 44.858 minutes is presented in **Figure 11.**

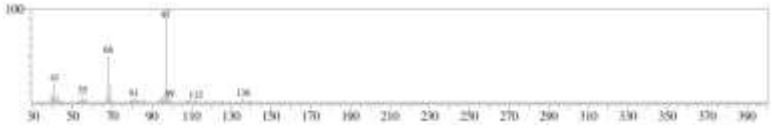


Figure 11. Mass spectra with Rt : 44.858 minutes

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Based on the mass spectrum Fig.11 shows the peak m/z: 98 is the peak characteristic of ether compounds by the presence of α (Gross, 2011)[9]. The peak is similar to the peak pattern of fragmentation of the cis-2-oxabicyclo,4.4.0-decane compound, in WILLEY229 library can be seen in **Figure 12.**

Figure12. Standart Mass Spectra of cis-2-oxabicyclo, 4.4.0-decane compound

The possible pattern of cis-2-oxabicyclo, 4.4.0-decane fragmentation is presented in **Figure 13.**

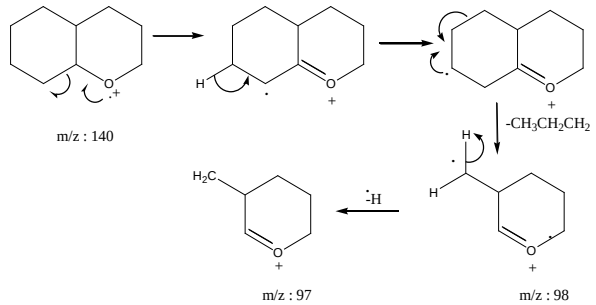


Figure 13.Hypothetical fragmentation pattern ofcis-2-oxabicyclo, 4.4.0-decane

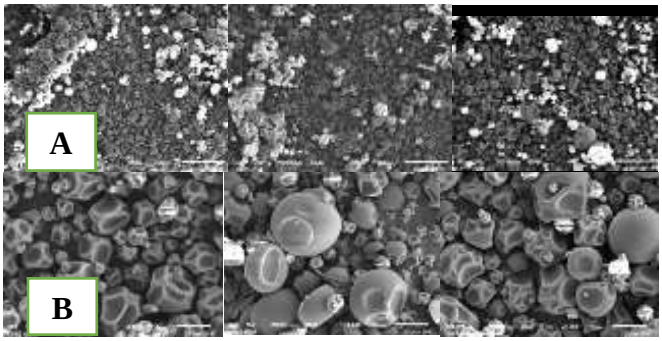
Based on the data that has been described, it can be tabulated the main components of essential oil of rogo presented in **Table 1.**

Table 1. Components of the main compound of essential oil of rogo

No	Compound	Rt (minutes)	Area (%)
1	Eugenol	27.307	47.89
2	Eugenyl acetate	32.738	9.13
3	Massoil	32.910	29.78
4	Cis-2-oxabicyclo,4.4.0-decane	44.717	12.35

3.1 Surface Morphology Analysis with SEM

Figure 14 presents photos of SEM results and microencapsulated rogo oil results as follows:



ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

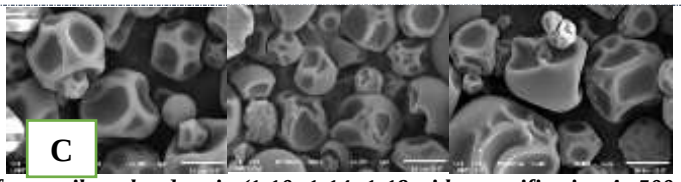


Figure 14. SEM results of rogo oil: maltrodextrin (1:10; 1:14; 1:18 with magnification A: 500x, B: 2000x and C: 5000x)

Figure 14 is a microencapsulated SEM rogo oil that is dragged according to the composition increasingly to the right, fewer rogo oil levels. Microcapsule form with ratio 1:10; 1:14; 1:18 have a resemblance or no significant difference. There is a small form of wrinkled balls on the appearance of the third SEM. The rounded particles intact indicate that the microcapsule is perfectly formed and contains rogo oil. The small form of wrinkled balls is thought to be the capsule of the capsules without the rogo oil therein or the less-perfect microcapsules (Elena and Mania, 2012)[10].

Rogo's Antibacterial Activity and Rogo's Microencapsulated Oil Result inhibiting E. coli Bacteria Growth

The results and analysis of antibacterial activity of rogo oil inhibiting *E. coli* growth can be seen in **Figures 15** and **Figure 16.**

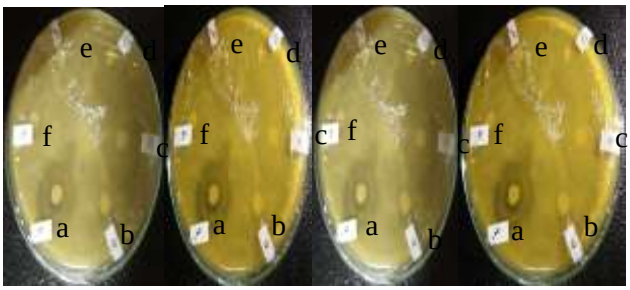


Figure 15. Result antibacterial activity test of rogo oil
Description: a. positive control, b. 100% essential oil of rogo, b. 50%, c. 25%, d.12,5% and e. negative controls

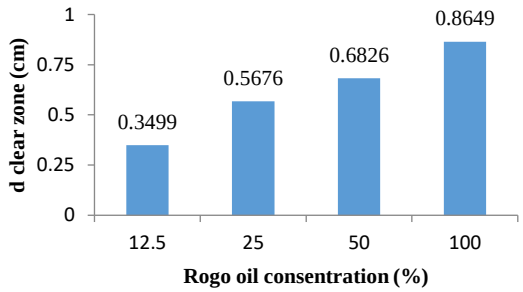


Figure 16. Analyzes of activity antibacterial test of rogo oil to E. coli bacteria

Figure 16 shows that in each variation of liquid rogo oil content there are different activities. Successive for concentration 12.5; 25; 50 and 100% provide activity expressed in clear zone diameter 0.3499; 0.5676; 0.6826 and 0.8649 cm. These data show that there is an increase in the clear zone as the rogo oil concentration increases. However, to see whether the difference in activity due to the increased concentration in the significant category or not, then t test. The t test results show that for concentrations of 100% and 50%, t value = 2.5737; Concentrations of 50% and 25%, tvalue = 1.8963 and concentrations of 25% and 12.5%, tvalue = 3.4346. While the value of t tab $\alpha = 0.05 = 3.18$. So it can be concluded that for the concentration of 100%, 50%, and 25% there is no difference, but different for the concentration of 12.5%. According to Ahmad (2013)[11] enhancement of zone transparency drag as the concentrate of essential oil increases.

ISSN: 2277-9655
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The activity of essential oils antibacterial depends on the component as well as the percentage of its constituent compounds (Nazzaro, 2013)[12]. The main compound of rogo oil compound is eugenol is a secondary metabolite compound of phenolic group that can give toxic effect on bacteria. In addition, the hydrophobic properties of eugenol make it easier to enter the lipopolysaccharide part of the bacterial cell membranes, especially the gram-negative bacteria and alter the cell wall structure, causing leakage in the intracellular part (Devi, *et al*, 2010)[13]. When the phenol group compound penetrates the bacterial cell membrane then interacts with enzymes and proteins in the membrane it can lead to adhesion of the bacterial cell membrane so that the osmotic pressure increases. It can cause damage to the cell membrane and inhibit the respiration of bacteria which in turn will cause disruption of ion transfer in the cell so that bacteria experience death. (Xu, Jian-Guo, et al, 2016 and Xu, X., 2012) [14][15].

The results and the microencapsulated analyzes of antibacterial show different activities as in **Figures 17 and 18**

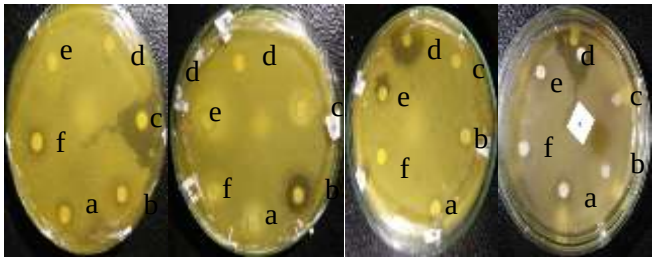

Figure 17. Result of activity antibacterial test of rogo oil microencapsulation to E. coli bacteria
Description: a. positive control, b. Essential oil rogo: maltodextrin 1:18; C. 1:16; d.1: 14; e.1: 12 and f.1: 10

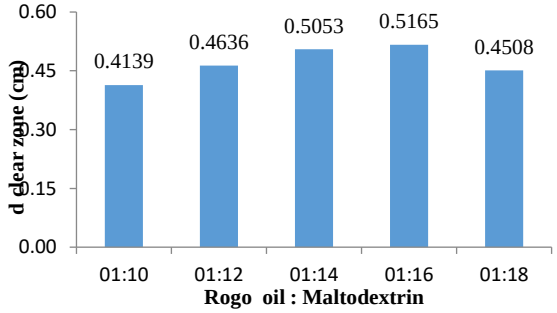

Figure 18. Analyzes of activity antibacterial test of rogo oil microencapsulation to E. coli bacteria

Successive for variations of maltodextrin 1:10, 1:12; 1:14; 1:16 and 1:18 provide the activity of a clear zone diameter of 0.4139; 0.4636; 0.5053; 0.5165 and 0.4508 cm. These data show that there is an increase in the clear zone as the coating material increases, but at 1:18 the concentration has decreased. The t test results show that for a ratio of 1:18 vs. 1:16 with t value = -1.5921; 1:16 vs 1:14 with t value = 0.1959, 1:14 vs 1:12 with t value = 0.8624 and 1:12 vs 1:10 ratio with t value = 0.7392; both> t tabs $\alpha = 0.05 = 3.18$. It shows that there is no significant difference of five treatment types. This situation occurs because the essential oil of rogo in this case as antibacterial activity can be said to be less effective because of the diameter of the clear zone <7 mm (Kumar, *et al*, 2014)[16]. So any comparison of rogo oil with maltodextrin as its coating will give effect not too different to E.coli bacteria.

When compared to the antibacterial test results of cloves where eugenol is also as the main compound as in Ahmad's study, *et al* (2013)[11] will be much different where the diameter of the clear zone is> 12 mm which can be said of its activity power. However, the antibacterial test of rogo oil has a difference with clove oil in terms of its constituent compounds. So it can be estimated that these rogo oil compound compounds have no synergistic effects in terms of their antibacterial activity.

The test results showed that for 100% rogo liquid concentration and microencapsulated 1:18; 1:16; 1:14; 1:12 and 1:10 in a t hit respectively ie 10.4526; 7,4158; 4.1320; 5,511 and 4,4916 which all have t value> t tab $\alpha = 0,05 = 3,18$. This shows that the average activity of 100% rogo oil and microencapsulated rogo based on the diameter of the clear zone gives the same result, where the antibacterial activity of rogo in liquid form (oil) is better than the microencapsulation result.

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IV. CONCLUSIONS

The main components of the rogo essential oil compound (*Premna serratifolia* L.) as antibacterial Escherichia *coli* based on characterization using GC-MS contain 4 main components such as eugenol (47.89%), eugenyl acetate (9.13%), massoil (29.78%), and cis-2-oxabicyclo, 4.4.0-decane (12.35%). %). The results of the rogo essential oil activity test showed that there was a decrease in concentration of 12.5%. While the result microencapsulated antibacterial activity test showed no difference from all variation concentration. The result of antibacterial activity test between rogo essential oil and microencapsulated results showed significant difference, the phase in the form of oil has greater antibacterial activity strength than the solid phase.

V. ACKNOWLEDGEMENTS

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