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Original Article

Effect of Using the Combination of Tween 80 and Ethanol on the Forming and Physical Stability of Microemulsion of Eucalyptus Oil as Antibacterial

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ABSTRACT

Objective: The purpose of this study was to determine the effect of increasing concentrations of the combination of tween 80 and ethanol to the formation and stability of the physical properties of eucalyptus oil microemulsion. **Methods:** This oil is formulated into a microemulsion by increasing the concentration of the combination of tween 80 and ethanol (1: 1) with various concentrations, ie 40%, 45%, and 50% as a component of surfactant and cosurfactant. The evaluation was done for 8 weeks covering organoleptic test, test phase separation, viscosity test, measurement of pH, density measurements, measurements of surface tension and particle size measurement. **Results:** The pH of micro-emulsion showed in the range 5.4 to 6.2. Viscosity results obtained viscosity values between 206.2 to 248 cps. Density measurement results were between 0.9839 to 0.9904 g/ml. The results of surface tension measurement results obtained between 35.83 to 37.10 dyne/cm. The results of particle size measurement results obtained from 23.83 to 30.43 nm. **Conclusion:** It can be concluded that the use of tween 80 and ethanol can form a microemulsion system of eucalyptus oil, and with increasing concentrations of the combination of tween 80 and ethanol can increase the stability of the physical properties of the micro-emulsion system.

Key words: antibacterial, ethanol, eucalyptus oil, micro-emulsion, stability of the physical properties, tween 80.

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INTRODUCTION

Empirically, eucalyptus leaves efficacious to relieve inflammation and pain (analgesic).[1] Extract of eucalyptus demonstrates their potential for the treatment and prevention of *S. pyogenes* induced disease, but the toxicity may limit their use to topical treatments for pharyngitis and impetigo.[2] Eucalyptus oil also showed better *in vitro* permeation on organogel formulation as compared to other penetration enhancer.[3] In a previous study showed that the use of 10% eucalyptus oil can also be active as an antibacterial.[4] To optimize its use, the eucalyptus oil is formulated in a microemulsion system. Microemulsion has many advantages compared to emulsions, among others, have stability in the long term by thermodynamics, clear and transparent, can be sterilized by filtration, the manufacturing cost is cheap, has high solubility and has good penetrating capability.[5] One of the most important components of a microemulsion forming was surfactants. The combined use of surfactant and co-surfactant can improve the dispersion of oil in water.

Surfactants are needed in the formation of oil in water microemulsion systems (O / W) is a surfactant with HLB value of 9-20, Tween 80 was suitable because it has a HLB 15.[6] Alcohol short to medium chain used in the microemulsion as cosurfactant, the use of ethanol as a cosurfactant in addition to improving the ability dissolves and can also function as penetration enhancers drugs. The concentration of surfactant and cosurfactant can determine the size of droplet sizes that can affect the stability of the physical properties of the microemulsion. Therefore, the research done to knowing the effect of increasing concentrations of tween 80 and ethanol (1:1) as a surfactant and cosurfactant on the formation and stability of the physical properties of eucalyptus oil microemulsion.

This study aims to determine the effect of increasing concentrations of the combination tween 80 and ethanol as a surfactant and cosurfactant with a ratio of 1:1 on the formation and stability of the physical properties of eucalyptus oil in the microemulsion.

MATERIALS AND METHODS

Eucalyptus oil were purchased from PT. MSU, Tween 80 were purchased from PT. KAO Indonesia Chemicals, Ethanol were purchased from PT. Stefani Dwi Tunggal, and Aqua destillata.

Determination of the Microemulsion Region (Figure 1)

Preparation of Eucalyptus Oil Microemulsion

Tween 80 was dissolved in aqua fervida until homogeneous using a magnetic stirrer (water phase). Eucalyptus oil was mixed into the water phase and then homogenized. Add ethanol drip-by-drip to form a clear and transparent of microemulsion. Stirring lightly to help improve homogeneity.

Evaluation of the Microemulsion

Organoleptic microemulsion was observed whether the color changes, changes in odor, clarity changes and other changes that may occur during storage.

Measurement of pH is done by using a digital pH meter,[7] i.e : calibration electrodes using the standard buffer solution pH 4 and pH 7, and then determine the pH value of the microemulsion.

Density was measured using a pycnometer,[8] i.e : empty pycnometer weighed at a temperature of 25° C (W_0), then weigh pycnometer filled with distilled water at a temperature of 25° C (W_1), and weigh pycnometer filled with the microemulsion at a temperature of 25° C (W_2).

Density = (W3 - W1) / (W2 - W1) (1)

Viscosity measurement is done by using a Brookfield viscometer. Microemulsion is inserted into a glass beaker, pair spindle No. 1 at 60 rpm.

Test Phase Separation Centrifugation and freeze thaw[9]

Centrifugation, microemulsion incorporated in a centrifuge tube and then centrifugation in speed of 3750 rpm for 5 hours, observe the changes that occur.

Freeze thaw done with the storage of 4°C followed by storage at a temperature of 45°C. Observe organoleptic changes, for 6 cycles. Where one cycle is stored at a temperature of 4°C for 24 hours, then transferred to a preheated oven at 45°C for 24 hours. Observable physical changes that occur.

Measurement of surface tension with du Nouy tensiometer. Place the sample to be measured correctly, note the number indicated by the instrument.

The average particle size,[10] was measured by using Particle Size Analyzer. The sample solution is inserted into the cuvette, cuvette filled sample is inserted into the sample holder.

Statistical analysis

The significance was determined by ANOVA. P<0.05 is considered statistically significant.

RESULTS

The Microemulsion Region

Based on these results, the microemulsion area obtained by the concentration of the combination of tween 80 and ethanol (1: 1) ≥ 39% (Table 1). Further concentration used for testing the stability of the physical properties at room temperature for 8 weeks is the concentration of surfactant and cosurfactant 40% (F1), 45% (F2), and 50% (F3). Concentrations were chosen because after storage at room temperature for 1 month, microemulsion did not have physical changes, and the surfactant concentration is still within safe limits the use of a tween, ie <50%.[11]

Organoleptic (Table 2)

Measurement of pH

All formulas produce a pH value that meets the requirements of the pH of the skin, which is in the range from 4.5 to 6.5 (Figure 2). Results of pH measurement for 8 weeks showed a decrease and an increase in pH which tend to be less significant during storage (sig. 0.670>0,05). From the measurement of pH is seen that with the increasing combination tween 80 and ethanol concentration causes a decrease in the pH value of a microemulsion, because the use of cosurfactant which is an alcohol with the chemical properties polihidric so easily oxidized. Alcohol oxidation produces carboxylic acid to reduce the pH value.[12]

Density

On the results of density measurements of microemulsion eucalyptus indicated that the increasing concentration of surfactant (tween 80) was tends to increase the density of the preparation. This is because tween 80 has a density of 1.070 g / mL at a temperature of 25°C. The higher of surfactant in the microemulsion formula, it will increase the density of preparations.[13] From the results of measurement of density, showed that the density of all the formulas were not too large which is between 0.9839 to 0.9904 g/mL so that the preparations can flow properly and easily pourable (Figure 3).

Viscosity

The highest viscosity values obtained in F3 (50%). This is because the use of surfactants in F3 is the highest that will make the dispersion medium becomes more rigid (Figure 4). The more rigid dispersion medium will result in increasing the viscosity of the microemulsion system.[14] The viscosity of a preparation affected by several factors including, the factor of mixing or agitation during the process of production, the selection of thickening agent and surfactant, the proportion of the dispersed phase, and the particle size.[15] The different concentrations of tween 80 in each formula and length of storage time affects the viscosity of dosage.

Test phase separation

Test centrifugation aims to determine the stability of the microemulsion after vigorous shaking. During storage, the microemulsion will have the force of gravity, according to stokes law that gravity can affect the stability of the microemulsion. Based on the results found that in F1 - F3 no phase separation occurs. This proves that a layer of surfactant and cosurfactant strong enough to protect the oil droplets so that no phase separation due to centrifugal force is given and this preparation is quite stable.

Table 1: Formula Microemulsion

No.	Oil Phase (%)	Combination of Surfactan - Cosurfactan (%)	Water Phase (%)	Physical Appearance
1	10	10	ad 100	cloudy
2	10	15	ad 100	cloudy
3	10	20	ad 100	cloudy
4	10	25	ad 100	cloudy
5	10	30	ad 100	cloudy
6	10	35	ad 100	cloudy
7	10	37	ad 100	cloudy
8	10	39	ad 100	transparent
9	10	40	ad 100	transparent
10	10	42	ad 100	transparent
11	10	45	ad 100	transparent
12	10	47	ad 100	transparent
13	10	50	ad 100	transparent
14	10	55	ad 100	transparent
15	10	60	ad 100	transparent

Table 2: Organoleptic of Microemulsion

Organo leptic	Formula	Time (Week)				
		0	2	4	6	8
Appearance	1	Liquid	Liquid	Liquid	Liquid	Liquid
	2	Liquid	Liquid	Liquid	Liquid	Liquid
	3	Liquid	Liquid	Liquid	Liquid	Liquid
Color	1	Canary	Canary	Canary	Canary	Canary
	2	Canary	Canary	Canary	Canary	Canary
	3	Canary	Canary	Canary	Canary	Canary
Odor	1	Specific	Specific	Specific	Specific	Specific
	2	Specific	Specific	Specific	Specific	Specific
	3	Specific	Specific	Specific	Specific	Specific

Table 3: Result of Freeze Thaw

Formula		Cycles 1		Cycles 2		Cycles 3		Cycles 4		Cycles 5		Cycles 6	
		4°C	45°C	4°C	45°C	4°C	45°C	4°C	45°C	4°C	45°C	4°C	45°C
F1	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F2	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F3	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-

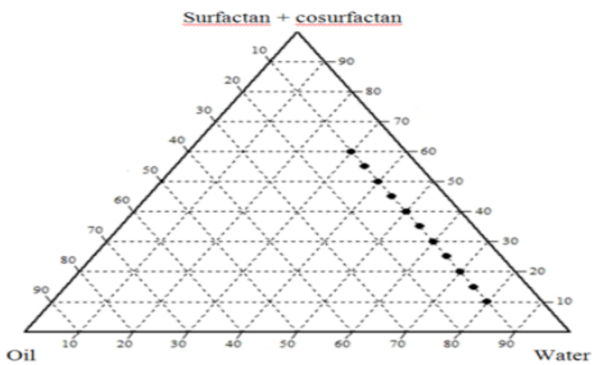

Figure 1: Diagram Determination of Eucalyptus Oil Microemulsion Regions.

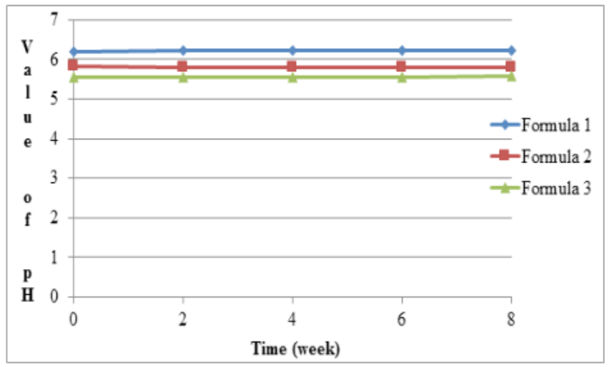

Figure 2: Results of pH Value.

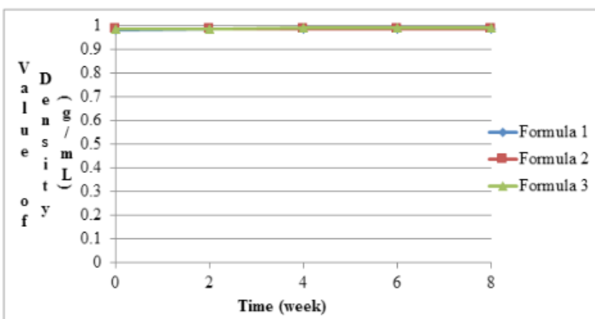

Figure 3: Results of Density Value.

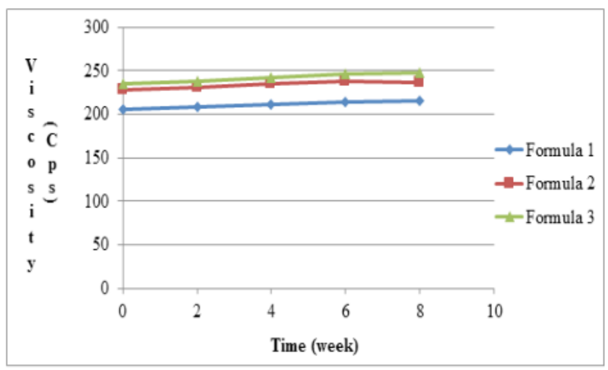

Figure 4: Results of Viscosity.

Results of Freeze Thaw cycles during storage preparation 6 cycles can be seen in Table 3. The test is performed at different temperatures at regular intervals so that the preparation in the container will experience stress vary. The test results showed that there was no change in F1 - F3 which means that each formula has remained stable against changes in temperature extremes.

Surface Tension

After the measurement was done for 8 weeks showed that the surface tension of the three formulas has increased (Figure 5). However, the increase are not too significant (sig. 0.455> 0.05). The addition of surfactants will cause a decrease in surface tension, the surface tension at a certain concentration will be constant even if the surfactant concentration increased. Cosurfactant added to help lower the interfacial tension of oil phase and water phase. Cosurfactant will form a microemulsion droplet thereby increasing the solubility of non-polar groups.

The Average Particle Size

The expected particle size is the particle size that ranges between 10-100 nm. Determination of particle size by this PSA showed that the resulting microemulsion has an average particle size of between 23.83 to 30.43 nm (Figure 6). An increase in particle size on a weekly basis, it could be due to the merging of small particles into a large group and also the distance between adjacent particles making it easier for particles to join together.

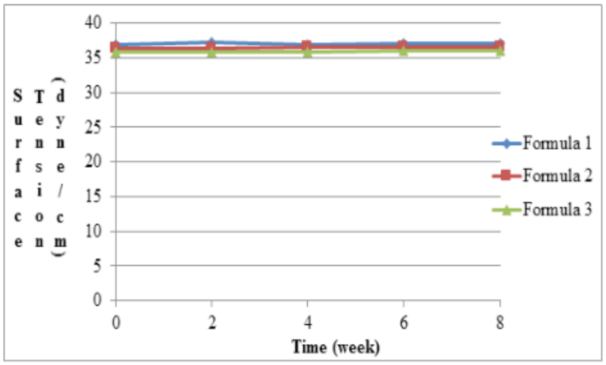

Figure 5: The Results of Surface Tension

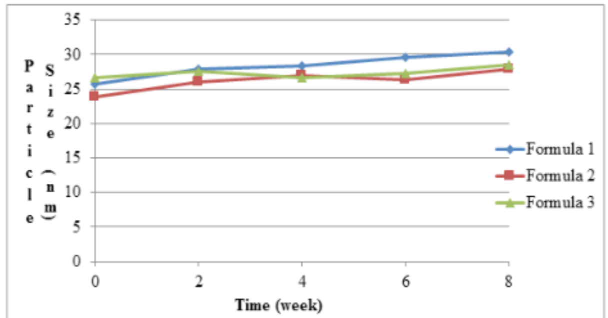

Figure 6: The Results of Particle Size

All formulas have a transparent appearance because the smaller of droplet size causes microemulsion reflect light that makes it look transparent. Small droplet size was also caused by the adsorption of surfactant at the interface between liquid.[6] Increasing the concentration of surfactant and cosurfactant will be able to decrease the size of the globules, this happens due to the increase adsorption of surfactant at the interface of oil and water, thus supporting the formation of a smaller globule size.[16] Based on statistical analysis of particle size results obtained sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.000 (<0.05). It can be concluded that the difference in concentrations of tween 80 and ethanol in any formula affects the particle size of the preparation, as well as the storage time affects the particle size of the microemulsion each week.

CONCLUSION

This paper has shown that by increasing concentration of surfactant and cosurfactant combinations can form a microemulsion system and improve the stability of the physical properties of the microemulsion system. This research finding show that eucalyptus oil can formulated to be microemulsion at 10% as active ingredient by using the combination of Tween 80 and ethanol (ratio 1:1) $\geq$ 39%. Concentration of the combination of surfactant and cosurfactant sufficient to reduce the interfacial tension between oil and water phase directly due forming the microemulsion.

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CONFLICT OF INTEREST

No conflict of interest from related parties involved in this research.

ABBREVIATIONS USED

ANOVA: Analyze of Variance; **F1:** Formula 1; **F2:** Formula 2; **F3:** Formula 3; **HLB:** Hydrophilic-Lyphophilic Balance; **O/W:** Oil in Water; **pH:** power of Hydrogen; **S.pyogenes:** Streptococcus pyogenes; **W:** Weight.

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