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Formulation and Antibacterial Activity Test *Staphylococcus epidermidis* as Microemulsion Preparation of Cherry Leaf Extract (*Muntingia calabura* L.) using the oil phase Isopropyl Myristate (IPM)

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ABSTRACT

Cherry leaf (*Muntingia calabura* L.) is one of the potential plants in the field of treatment, one of them as antibacterial in the treatment of anti-acne. Cherry leaf contains flavonoid compounds, saponins and tannins. This research is about the formulation of microemulsion of cherry leaf extract in improving penetration drug capability into skin.The purpose of this research is to know microemulsion chemistry and physic characteristics of cherry leaf extract and to know which one is the good formula based on microbial activity test of cherry leaf extract to *Staphylococcus epidermidis*. The results of this study showed that the microemulsion of cherry leaf extract had good characteristics on F2 with the concentration of 10% cherry leaf extract with clear brownish yellow color, peculiar leaf of cherry leaf, had pH value 5,94, type of microemulsion was oil in water (o/w) with the particle size was 15.01 nm, had an index polydispersity value of 0.22 <0.5, and can inhibit the growth of *Staphylococcus epidermidis* with antibacterial activity result was 8.34 ± 0.13. The microbial activity can be categorized as intermediate. Antibacterial activity using cendoxitrol as a positive control has an activity of 16.9 ± 0.51 with strong category.

Keyword:

Microemulsion
Cherry Leaf Extract *(Muntingia calabura* L.*)*
Antibacterial,
Staphylococcus epidermidis

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1. INTRODUCTION

Natural ingredients are currently more used because they have lower side effects than synthetic drugs or chemicals. Moreover those are more affordable in terms of price, and the raw material is easily found. Cosmetics and medicines that use natural substances as active ingredients of products are now a day'strendings. These continueslygrow with concept of*"backto*nature"which use the plants of local wisdom, especially in Indonesia, which has potential of naturalmedicines and cosmetics [1]. One of plants that has a potential ascosmetics especially foranti-acne is cherry*(Muntingiacalabura* L.) particularly used in the leaves.

Cherry leaves are known containing flavonoids, saponins, steroids, triterpenoids and tannins that can be potentially used as antibacterial in the treatment of acne [2]. The treatment of acne in skin clinics recently uses antibiotics to inhibit inflammation and kill bacteria, but the use of antibiotics in the long term can lead to resistance and can cause organ damage and immuno hypersensitivity [3]. Those effects can be prevented by using natural materials that are relatively safe and have lower side effects than synthetic materials.

Development of antibacterial formulation of cherry leaf hopefully can be used the public use.So, it needs to be formulated in dosage forms of topical preparations in which more easily penetrate into the dermis asamicroemulsion.

Microemulsion is a drug delivery system that has potential to increase the penetration of the drug penetrating to epidermal layer because it has a size of 0,1-100 μm.To accelerate the microemulsion penetrating to the layers of human skin it is composed by an oil in water phase.The aqueous phase is mixed by a surfactant and cosurfactant, because surfactant and cosurfactan can be easily adjusted in the manufacture of microemulsion so it can reduce the barrier of diffusion of the stratum corneum which acts as a enhancer [4].

2. METHODS

2.1 MATERIALS RESEARCH

The materials used in this study were cherry leaves*(Muntingia calabura* L.),*Nutrient Agar* (NA) media, *Luria Bertani Broth* (LBB) media, Ethanol 96% (Pt. Bratachem), PEG 400 (Pt. Bratachem), Tween 80 (Merck) Span 80, Isopropyl myristate, Cendoxitrol eye drops, *Methylene red*, sulfuric acid, acetic acid, potassium dichromate, magnesium (Mg), HCL acid, $FeCl_3$, DMSO, NaCl, Aquadest, *Pharmaceutical grade.*

2.2 RESEARCH TOOLS

The tools used in this study consisted of digital scales (Shimadzu Union Bloc), glass measuring 100 mL (pyrex), rod stirrer, Rotary evaporator (IKA RV 10 Basic), Sonica® Ultrasonic Cleaner, 100 ml glass beaker (pyrex), Erlenmeyer 500ml (pyrex), *Particle Size Analyzer* (PSA), *Laminar Air Flow* (Minihelix II), petri dish, Pipette drops, hotplate, micropipette 10ml, ose needle, Autoclave, Incubators, Bunsen, test tube (pyrex) , pH meter type 510 (Eutech Instrument), Oven (Memmert UN 55), a test tube (pyrex), a spray bottle, cupboard Cooling (LG), magnetic stirrer (IKA RW 20 Digital), calipers, *Whatman* paper,*blue-type*

2.3 Preparation cherry Leaf Extract *(Muntingia calabura* L.)

Plants were obtained from the MateriaMedikaBatu as much as 1 kg.500 grams of cherry leaves were extracted using ethanol 96% with *ultrasonic cleanser* for 18 minutes at a ratio of 1:10 (w/v), then the filtrate was evaporated with *a vacuum rotary evaporator* to obtain a thick extract.

2.4 Free Test Identification of Active Compounds Ethanol and cherry leaf

Ethanol-free test was done by two methods, esterification of ethanol and the reduction and oxidation reactions. Further test of active compound identification includes examining cherry leaf extract flavonoids, tannins and saponins.

2.5 Microemulsion Formulation Cherry Leaf Extract *(Muntingia calabura* L.)

Table 1. Formulation of Microemulsion Cherry Leaf Extract *(Muntingiacalabura* L.) with the 3 replication

Ingredients	Function	Concentration			
		F1	F2	F3	F4
cherry leaf extract	active ingredient	5%	10%	15%	-
IPM	oil phase	5%	5%	5%	5%
Tween 80	surfactant	33.5%	33.5%	33.5 %	33.5%
Span 80	surfactant	16.5%	16.5%	16.5 %	16.5%
PEG 400	cosurfactant	5%	5 %	5%	5%
Aquadest	Solvent	100%	100%	100%	100%

2.6 Preparation of Microemulsion system Leaf Extract cherry and Physical Evaluation

Microemulsion formulation in Table 1 is based on the calculation of the equilibrium value of HLB. First microemulsion phase was formulation of aqueous phase consisting of tween 80 and water-free CO_2 stirred by *a magnetic stirrer* at 500 rpm for 15 minutes, and then the oil phase consisted of span 80, PEG 400, IPM and cherry leaves extract stirred at 500 rpm for 15 minutes. Then the two phases were mixed into one using *a magnetic stirrer* at a speed of 1000 rpm at a temperature of 50˚C to form a homogeneous and clear microemulsion.

Evaluation of cherry leaf extract microemulsion system was done by testing the organoleptic characteristics, pH test, stability freeze-*thawtest*, the determination of emulsion type, humidity test and measurement of particle size.

2.7 Antibacterial Activity test *Staphylococcus epidermidis*

Antibacterial activity test used 6 treatments: positive control, negative control and cherry leaf extract in three concentrations (5%, 10% and 15%). The test was done using well diffusion method.

3. RESULTS AND DISCUSSION

3.1. Preparation cherry Leaf Extract *(Muntingia calabura* L.)

Simplisia cherry leaves were extracted with 96% ethanol using ultrasonic extraction method for 18 minutes with a ratio of 1:10 (w / v), obtained liquid extract was then evaporated with a *vacuum rotary evaporator* to obtain thick extract. Final extract was blackish solid green viscous with total weight 80 grams of ethanol extract from leaves of cherry 16%.

3.2. Free Test and Identification of Active Compounds Ethanol

ethanol-free test was conducted to ensure cherry leaves are free from ethanol. Because ethanol itself is an antibacterial and antifungal that can affect the antibacterial activity test. Cheery leaf extract was free from ethanol proven by the change when sulfuric acid was added to potassium dikarbonatresulting a brown color. There is no evidence of ester smell from the reaction of esterification of ethanol between acetic acid and sulfuric acid in cherry leaf extract assisted by heating. The reacton is shown in the figure below

$$K_2Cr_2O_7 + 4H_2SO_4 + 3CH_3CH_2OH \longrightarrow Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O + 3CH_3CHO$$

Figure1. Redox Reactions

$$CH_3\overset{\overset{O}{\|}}{C}OH + HOCH_2CH_3 \rightleftharpoons CH_3\overset{\overset{O}{\|}}{C}OCH_2CH_3 + H_2O \quad (H^+_{kalor})$$

Figure2. Ethanol Esterification Reaction

Identification of active compounds was done qualitatively. The aim is to determine the content of the active compounds in the leaves of cherry, including flavonoids, tannins and saponins. The results of the identification of active compounds botanicals are shown in **Table 2.**

No	Secondary of Metabolic	Reagent	indicator	Results
1.	Flavonoids	Mg,concentratedHCl	orange color	+
2.	Tanin	FeCl3	blackish green color	+
3.	Saponin	Aquadest, 2N hydrochloric acid	was formed froth ± 1.5 cm	+

Identification of Flavonoids

Identification test of flavonoid compounds showed orange discoloration after the addition of Mg and concentrated HCl. According to Prashant *al*.[5] the addition of concentrated HCl reduces the existing core on the structure benzopironflavonoids. It is a sign of positive flavonoids in cherry leaf.

Identification of Tannins

From the analysis that has been done, it can be seen that in a positive cherry leaf extract contains tannins. This is evidenced by a color change to green-black, according to Harborne [6] the formation of blackish green after $FeCl_3$ solution was added is because the tannins can react with $Feions^{3+}$to form a complex compound.

Identification of Saponin

From the analysis showed that cherry leaves extratctcontainsaponins evidenced by the formation of foam as high as ± 1.5 cm after adding distilled water and shaken vigorously. The emergence of foam in the test show their saponin glycosides that have the ability to form foam in water which can be hydrolyzed by glucose and aglycone [7].

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3.3 Physical Evaluation of Microemulsion cherry Leaf Extract

3.3.1 Organoleptic test

Based on organoleptic examination held for 8 weeks in the three formula showed that microemulsion were stable when these were stored at room temperature (27˚C). Each formula has almost similar organoleptic characteristics except the color of the microemulsion.Itis because by increasing the cherry leaf extract concentration the microemulsion was getting browned. Besides the increase in the concentration of cherry leaf extract tend to improve the consistency of the microemulsion, the formulation at concentration of 5% has the most watery consistency, whereas the concentration of 15% has the consistency of slightly viscous and had a distinctive smell of cherry leaves.

Figure 3. Physical Appearance microemulsion with cherry Leaf Extract Concentrations of 5%, 10% and 15%

3.3.2 Test pH

The pH value of three formula decreased at fourth and fifth week but the difference has no effect because they were at a pH range 4,5-6,5. This range according to Annisa, R, et all., [8] is ideal for topical because it is skin pH. The formulations does not indicate any changes in the pH testso it can be declared as stable.

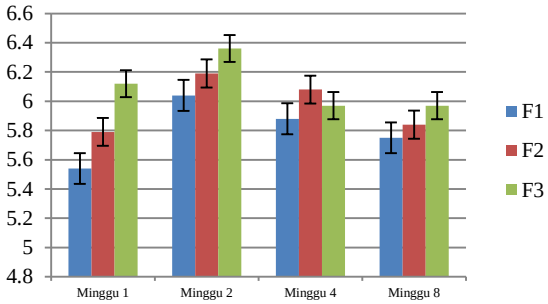

Figure 4.the charts the average pH value examination

3.3.3 Freeze-*thaw* Stability Test

Freeze-*thaw* was used to determine the microemulsion of cherry leaf extract precipitation or phase separation so that the stocks can said as thermodynamically stable.This reaction is *reversible* or other wise. freeze-*thaw* stability tests performed on the formulations at different temperatures that are cold temperature (4˚C) and high temperature (40˚C) for 6 cycles.

The result of in all three formulations showed that there were physical changes occurring in the 2nd cycle when they were stored at cold temperatures to freeze, the F1 had a yellow color, F2 had a canary yellow and F3 had a brownish yellow color but when microemulsion was transferred at room temperature (29ºC) and high temperature (40˚C) the texture back to stable forms and the original color, it indicates that the oil and water phase with the add of surfactant to form a single solution was dispersed well and *reversible* since no phase separation occurs.

3.3.3.1 pH Test Before and After Stability Freeze-*thaw*

pH measurement of formulations before and after stability freeze-*thaw*showed that the average pH of the microemulsionof F1 was 5,79 ± 0,16; F2 was 6,23 ± 0,07 and F3 was 6,09 ± 0,45. The *Paired t-test* at a level of 95% of the formula II obtained significance value of 0,00 (P <0.05), which means there was a significant difference

between the formula with pH values. This is due to an increase in temperature without using a concentration of 10 % will result in an increase in pH.

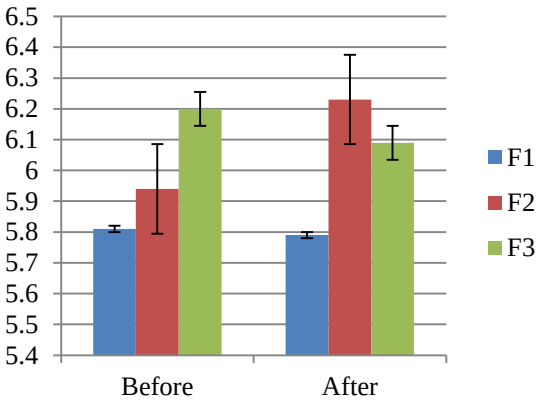

Figure 5. Average Test Results Before and After the pH value Stability

Test Antibacterial Activity

Test results of antibacterial activity of *Staphylococcus epidermidis* with cherry leaf extract concentration of 5%, 10% and 15% was demonstrated with the formation of a clear zone around thehole sinks. Each replication was performed 3 times, the goal is to get more accurate results. Clear zone resulting of concentration of 5% produced in average 6,13mm, microemulsion with 10% extract concentration was 8.34 mm , and micro emulsion with 15% extract concentration produced an average clear zone of 12.2 mm. The positive control cendoxitrol clear zone was 16.9 mm and a negative control as well as control the comparison resulted in no clear zone (0 mm). It can be concluded that the higher the concentration, the greater the diameter of the clear zone around the hole.Addition to the results obtained related to the inhibition zone standards according to Davis and Stout [9] that have an average diameter of inhibitory zone was included in the category of medium and strong.

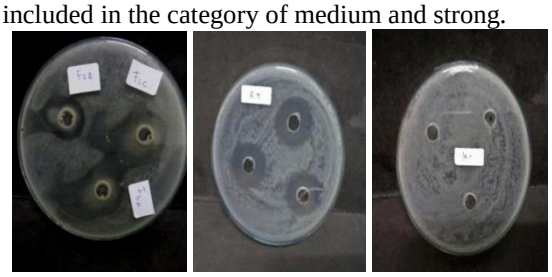

Figure 6. Results of Antibacterial Activity Test *S.epidermidis* extract concentration of 15%, a positive control and a negative control.

A clear zone around the hole wells is caused by the content of the compounds of cherry leaf extract that are flavonoids, saponins and tannins. These have mechanism of action of the antibacterial.

To determine whether there is a significant difference between the groups, statistical analysis was done by *One-way* ANOVA. It showed that microemulsionofcherry leaf extract can inhibit bacterial growth inhibitory S.epidermidis with an average diameter different ($p < 0.05$). Further testing of LSD showing three formulas had no significant differences between the positive control group and the treatment. It is because the positive control has a broad spectrum so it can fight the bacteria both Gram-positive and Gram-negative better than the extract. Showed by that diameter zone of inhibition produced by the positive control waslarger than the inhibition zone produced by cherry leaf extract.

3.3.3 Examination of Particle Size Microemulsion

The examination of particle size was done to F2 with a concentration of 10%. this is due to the 10% have a good physical evaluation results of organoleptic tests, pH,freeze-*thaw* stability and bestconcentrationantibacterial activity compared with 5% and 15%.In addition the concentration of 10% had a good inhibitory ability of 8.34 ± 0.13. It had the medium categoryinhibiting*S.epidermidis*bacteria.So 10% concentration was selected as effective formula for the examination of particle size.

The result ofmicroparticles have an average particle size of 15.01 nm with a polydispersity index of 0.22<0.5. It was considered to have a narrow size distribution. It can be concluded that the micro emulsion cherry leaf extract has a good particle size homogeneity. Besides the material components contained in the micro emulsion affect

particle size, as shown by the high concentration of surfactant used in the preparation resulting in many formation of micelles [10].

Figure 7. Results of Measurement Cherry Leaf Extract Microemulsion with PSA

4. CONCLUSIONS

1. Formulation of microemulsion cherry leaf extract*(Muntingia calabura* L.) had the physical characteristics of both organoleptic chemical, having a pH range of 5-6, microemulsion type oil in water (O/W), average 15.01 nm particle size, and the formulation remained stable during the period of storage meet the requirements of pharmaceutical standarts.
2. The best Formulation of microemulsion cherry leaf extract after the evaluation of chemical physics characteristics with broad zones of inhibition of 8.34 ± 0.13 mm was obtained in F2 with a concentration of 10%.

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