Influence of CMC-Na Concentration to Physical Properties of SNEDDS Kersen leaves Ethanolic Extract Gel

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Abstract: Kersen leaves (Muntingia calabura L.) consist of flavonoid, saponin and tannin that has bacteriostatic activity to bacteria caused acnes such as Staphylococcus epidermidis. In this research the extract is loaded into SNEDDS [Self Nano Emulsifying Drugs Delivery System] in order to optimize the absorption of active substances because of the lipophilic character of stratum corneum. SNEDDS then dispersed into hydrogel matrix in order to help application on skin. Kersen leaves extracted using ethanol 96 % by maceration method. SNEDDS formula composition are candlenut oil, tween 80 as surfactant and PEG 400 as cosurfactant in ratio 1:6:1, that contained 30 mg extracts each system. The hydrogel matrix made with carbopol with variation concentrations: 0.06 %; 0.13 % and 0.19 %, then physical property test: organoleptic, homogeneity, pH, viscosity, separation, adhesive and cycling test. Data were analyzed using One Way ANOVA to know the influence of variation of CMC-Na concentration. The results showed that SNEDDS Kersen leaves Ethanolic Extract with 0.06 % carbopol concentration indicated an optimum formula based on physical stability tests. Statistical analysis of the variation of carbopol concentration showed a significant difference on physical characteristics there are: viscosity, pH, homogeneity, and separation test.

1. Introduction
Acnes usually can be treated with antibiotics. According to Utami [1] the long term use of antibiotics causing microbial resistance, also cause organ damage and immunohypersensitivity, so that we need to develop acne treatment using traditional plants as active ingredients. One of the plants that can be used is kersen leaves. Based on Handayani’s research [2], ethanolic extract of kersen leaves has bacteriostatic activity against Staphylococcus epidermidis which is a bacterium that causes acne at concentration of 9,0 ppm and has diameter of inhibitory 14.0 mm. Nanoemulsion is one of pharmaceutical dosage form that choose to develop ethanolic extract of kersen leaves. Nanoemulsion has small particle size (200 - 300 nm) so can speed up the process of dissolving active substances of ethanolic extract of kersen leaves into skin [3], and also causes the drug to penetrate micron-sized bacterial cells more effectively. SNEDDS (Self Nano Emulsifying Drugs Delivery System) consist of the composition of oil, surfactants and cosurfactants [4]. Candlenut oil is chosen as an oil phase because it has better solubilization ability of hydrophobic drugs [5]. The selection of tween 80 as a surfactant because of its stable and have no toxicity and PEG 400 as a cosurfactant aims to create higher HLB (Hydrophilic Lipophilic Balance) values that meet the requirements in making
SNEDDS [6]. Gel preparations with extracts as active ingredients have been found, but have a less aesthetics appearance due to the brown color of the extract. That causes the preparation to be less pleasant when applied to the skin. SNEDDS ethanolic extract of kersen leaves that dispersed into hydrogel is one solution because it is able to produce clear appearance and the effectiveness of active substances to get the action target into skin. Carbopol is polymer that widely used as hydrogel matrix. Research of Kumar and Kumar, 2011 [7] shows that the physical properties of gel preparations that use carbopol as gelling agent are better than HPMC and CMC-Na. The basis for choosing carbopol concentration in this study refers to Djajadisastra, 2009 [8], which states that the optimal concentration of carbopol is 0.13-0.18 % with the concentration of propylenglycol as humectant between 5-9 % [6]. Based on the description above, SNEDDS ethanolic extract of kersen leaves (Muntingia calabura L.) were dispersed on hydrogel with variations carbopol concentration of 0.06 %; 0.13 %; and 0.19 %, and the physical properties of the preparation were tested which included organoleptic, homogeneity, pH, adhesion, dispersion, viscosity and stability test.

2. Experimental

2.1. Materials

GenesysTM 10s UV/VIS spectrophotometer, moisture analyzer, **rotary evaporator** (STUART RE300DB), pH meter (LUTRON PH-208), drop pipette, beker glass, **vortex** (MAXI MIX II), **sonicator** (DSA50–GL1-1.8L), **viscosimeter** (RION VT-04/03), stopwatch, water bath, Prescia BJ 4501C and Sartorius BP 221S analytical balance sheet, mikroskop (Nikon E100), centrifugation (HERAEUS FRESCO 17 CENTRIFUGE), **refrigerator** (LG).

Etanol 96 % (Bratachem), HCl (Merck), Mg powder (Merck), FeCl₃ (Merck). Candlenut oil (CV. Agung Jaya), Tween 80 (Merck), PEG 400 (Merck), Carbopol (Bratachem), Gliserin (Cusson), TEA (Bratachem), Propilenglikol (DOW), Metil Paraben (Bratachem).

2.2. Methods

2.2.1. Preparation ethanolic extract of kresen leaves

Kersen leaves taken from Cepu, Blora, Central Java, Indonesia. The selected leaves are old leaves because they contain more active compounds. Kresen leaves of 2.0 kg were washed with clean water, then dried into oven at temperature of 40 – 60 °C for 1 hour. The dried leaves then powdered into a smaller size by hand. Kersen powder of 300 grams, masetaed with 96 % ethanol until the surface of the powder was completely submerged, left for 3x24 hours with several times stirring. Maserate was filtered, then evaporated with a rotary evaporator and concentrated with waterbath.

2.2.2. Detection of active component kersen leaf ethanolic extract

The thickened extracts were tested for moisture content with Moisture analyzer, rendemment was calculated, and phytochemical qualitative tests included flavonoid, saponin and tannin tests. Test for flavonoids : by dissolving the sample into 2.0 mL 96 % ethanol solvent, adding a few drops of HCl and Mg powder. A positive result if it shows radish/orange colour of extract. **Tanin Test** : by adding FeCl₃, 1 % reagent to the extract, positive results if formed in black or greenish brown. Saponin test : by dissolving the sample into aquadest, and shaking it. Positive results if a stable foam is formed which does not disappear after 10 seconds.

2.2.3. SNEDDS formulation and Hydrogel

SNEDDS formulas of Kersen Leaves Ethanol Extract (Muntingia calabura L.) consists of oil phase: surfactant: co-surfactant with optimum ratio of 1: 6: 1 and extract content of 30 mg for every 1.0 g of system. The ingredients are weighed, then homogenization using vortex for 1-2 minutes, then incubated at 45 °C for 10 minutes. The SNEDDS formed underwent transmittance tests and centrifuged at 3000 rpm for 15 minutes at room temperature. The Hydrogel formula of SNEDDS at Table 1.
Hydrogel formulation made of 100 g. The hydrogel base is made using carbopol that dispersed into warm aquadest, adding TEA while stirring homogeneously. Methyl paraben is dissolved in glycerin, then mixed in a hydrogel base. Propylene glycol is added, stirred homogeneously, added with distilled water ad 100 % and added 2 g of SNEDDS, stirred ad homogeneously.

Table 1. Hydrogel formula of SNEDDS Kersen ethanolic extract with variation concentration of CMC-Na

| Ingredients (mg)                              | % w/w | F1 | F2 | F3 |
|----------------------------------------------|-------|----|----|----|
| SNEDDS concist extract 30 mg/system           | 2     | 2  | 2  |
| Carbopol                                     | 0.063 | 0.125 | 0.188 |
| Gliserin                                     | 10    | 10 | 10 |
| Propylene Glycole                            | 15    | 15 | 15 |
| Triethanoamine                               | 1     | 1  | 1  |
| Methyl Parabene                              | 0.1   | 0.1 | 0.1 |
| Aquadest                                     | ad 100| ad 100 | ad 100 |

2.2.4. Physical test of SNEDDS Kersen Leaf Ethanolic Extract (Muntingia calabura L.)

Physical test including organoleptic test, homogeneity, pH, adhesion, dispersion, viscosity and cycling test. Tests were carried out at 0, 14 and 28 day.

2.3 Data Analysis

Shapiro-Wilk test using SPSS 21 Software and data that normal distribution continue analysed with One-Way ANOVA to get significant information. Significant information data of One-Way ANOVA then continue analysed Post HOC test.

3. Results and Discussion

The results of the determination in the Biology Laboratory of the Mathematics and Natural Sciences Faculty, Sebelas Maret University Surakarta, Indonesia based on Flora of Java book written by C.A Backer and R.C. Bakhuizen van den Brink, Jr. (1963, 1968) stated that the plants used in this study were kersen leaves (Muntingia calabura L.). The result of kersen leaves ethanolic extract was 26.30 g with a yield value of 8.76 %. The results of the water content test showed that the thickened moisture content of the extract was 14.95 %, where the requirements of this water content had met the specified requirements, namely for thick extracts containing 5-30 % [9]. Organoleptic test results of kersen leaves ethanolic extract are dark green with the characteristic smell of kersen leaves and thick consistency. Phytochemical content test was carried out on extracts and SNEDDS and gel following results at Table 2.

Table 2. Phytochemical qualitative test of kersen leaves ethanolic extract and SNEDDS kersen leaves ethanolic extract

| Sample test | Phytochemical qualitative test |
|-------------|--------------------------------|
|             | Flavonoid | Saponin | Tanin |
| extract     | +         | +       | +     |
| SNEDDS      | +         | +       | +     |

3.1. Transmittance Test and Centrifugation Test on SNEDDS

SNEDDS formula was selected based on a trial of several formula comparisons, and the formula was obtained by comparing the composition of candlenut oil: tween 80: PEG 400 was 1: 6: 1 with an extract content of 30 mg (every 1.0 g of SNEDDS). The amount of extract content has been in
accordance with the literature, and has been able to provide bacteriostatic effects on the bacteria *Staphylococcus epidermidis*. The transmittance test on the selected formula shows the transmittance rate is 92.19%. The results of particle size test using PSA showed that the droplet extract size in SNEDDS was 15.2 nm. Centrifugation tests were carried out on SNEDDS. The result is that there is a phase separation with a separation ratio (F) value of 0.92. In several studies, an accelerated stability test was used to shorten observation time. The right centrifugation force will cause oil separation by damaging the emulgator layer absorbed around each grain. The more stable the emulsion is the greater the centrifugation force needed to damage the emulsion layer [10].

3.2. Physical test of SNEDDS Hydrogel of Kersen Leaves Ethanol Extract (*Muntingia calabura* L.)
Observation of physical properties was carried out at 0 day, 14 day and 28 day. There are: Organoleptic testing of SNEDDS hydrogel aims to determine organoleptic changes during storage at during 28 days. Organoleptic testing includes observation of the color, smell, and consistency of the hydrogel. The results of organoleptic observations are presented in Table 3.

The test results showed that the hydrogel formulation in three formulas did not show organoleptic changes. This shows that the hydrogel formulation is stable at room temperature storage. The difference in carbopol concentration affects the level of consistency of gel preparations.

Homogeneity testing aims to find out that all ingredients are mixed homogeneously. SNEDDS hydrogel of kersen leaves ethanolic extract showed that all formulas were homogeneous and did not change during the storage period. All formulas show evenly dispersed particles. It can be concluded that the variation of carbopol gelling agent concentration on SNEDDS hydrogel formulation does not affect the homogeneity.

### Table 3. Organoleptics test of SNEDDS Hydrogel of kersen leaves ethanol extract during 28 days for Formula 1, 2 and 3

| Formulas | Organoleptic test | Day to- | 0 | 14 | 28 |
|----------|-------------------|---------|---|----|----|
| F1       | Consistency       | Viscous | Yellow Brown | Yellow Brown | Yellow Brown |
| carbopol | Colour            | Kersen leaves | Kersen leaves | Kersen leaves |
| 0.06 %   | Odor              |         |               |               |               |
| F2       | Consistency       | Thick   | Yellow        | Yellow        | Yellow        |
| carbopol | Colour            | Kersen leaves | Kersen leaves | Kersen leaves |
| 0.13 %   | Odor              |         |               |               |               |
| F3       | Consistency       | Solid   | Clear yellow  | Clear yellow  | Clear yellow  |
| carbopol | Colour            | Kersen leaves | Kersen leaves | Kersen leaves |
| 0.19 %   | Odor              |         |               |               |               |

3.2.1. Viscosity test
Viscosity testing aims to determine the level of viscosity hydrogel that produced. A good gel is not too liquid or not too thick. Viscosity test results are presented in Figure 1. The viscosity values of optimum gel formulation are 2000-4000 cps [11]. The viscosity test results show that only formula I meets viscosity requirements. Based on observations it is known that the viscosities value of all formula have decreased. This can be caused by an increasing temperature, where the heat obtained will increase the distance between molecules so that the force will decrease and the distance becomes tenuous. Based on the normality test with the Shapiro-Wilk method during storage time showed that the viscosity data of formula I, formula II and formula III were not normally distributed (Sig <0.05). One Way Anova test cannot be done, because the data is not normally distributed. Based on the results of viscosity testing it is known that the higher concentration of carbopol influence the viscosity of the gel.
3.2.2 pH test

pH testing aims to determine the pH value of the hydrogel that produced. The pH requirements that do not irritate the skin are between 5-9 [6]. The pH of an overly acidic preparation can irritate the skin, whereas if the pH is too alkaline it can make the skin dry. The pH test results are presented in Figure 1. In observations every 14 days it is known that the pH of each formula has decreased. Changes in pH values can indicate a reaction or damage to the constituent components in the formulation, it caused may the hydrogel that used carbopol, so that it can reduce the pH value [7]. The results of ANOVA test between formulas showed that the pH of formula I, formula II, and formula III experienced a significant difference (Sig = 0.000). Post Hoc follow-up tests were conducted to find out which groups gave significant differences and obtained the results that the formula I, formula II and formula III each had a significant difference.

![Graph of Viscosity Test](image1)

![Graph of pH Test](image2)

![Graph of Spread Test](image3)

**Figure 1.** Diagram of physical properties test of SNEDDS hydrogel of kersen leaves ethanol extract includes viscosity, pH and spread test during 28 days. Highest concentration of CMC-Na caused viscosity and pH decrease each weeks, and increase the spread test.

3.2.3 Cycling Test

Cycling test of hydrogel formula conducted for 6 cycles shows that formula I, formula II and formula III stable organoleptically and Hydrogel formula not separate likes syneresis process. Syneresis is the process where a liquid is separated from the gel, it cause when gel placed too long or
stored in the open air, and the temperature rises [8]. This results in contraction in the gel mass. The entangled liquid will come out and be above the surface of the gel. Based on the results of the cycling test, it was concluded that the gel formula of SNEDDS stable the active component of kersen leaves ethanolic extract when stored at cool temperatures (2-8 °C) and room temperature (25 ±2 °C).

3.2.4 Spread test

Spread power test aims to determine the ability of the gel formulation to spread when applied to the skin. Spread test results are presented in Figure 1. The optimum spread of gel formula is between 5-7 cm³. Based on this literature, the spread value that meets the requirements only in formula I. Observations at day 0, day 14 and day 28 indicate that the dispersion power of each formula has decreased. During storage there can be a decrease in dispersion due to the retention of solvent which is absorbed by the gelling agent [5].

4 Conclusion

Kersen leaves ethanolic extract have flavonoid, tanin, and saponin compound. Based on the evaluation of the physical properties test of SNEDDS hydrogel of kersen ethanolic extract that increasing the concentration of carbopol as a gelling agent causes an increase viscosity and a decrease in dispersion and pH values. Formula I with concentration of carbopol 0.06 % shows the best results, because it meets the main requirements as a optimum gel formula.

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