Influence of formulation on physical stability of cherry (Muntingia calabura L.) leaves extract as an antibacterial

A Sultan* and F A Parumpu
Department of Pharmacy, Faculty of Sciences, Tadulako University, Kampus Bumi Tadulako, 94118, Palu, Indonesia

*Email: asriana.sultan@gmail.com

Abstract. Cherry leaves have been known as an antibacterial and a natural antiseptic that contain flavonoids, saponins and tannins. This research aims to prepare two formulation of cherry leaves extract and to evaluate their physical stability as an antibacterial dosage form. cherry leaves were extracted using ethanol 96% by maceration method. The extract was formulated as a hydrogel with carbomer and a water-based cream with combination of stearic acid and TEA. Physical stability was determined at cool temperature (4°C) and room temperature (±25°C) by organoleptic, pH, homogeneity, spread, and emulsion type test (for water-based cream). The results showed that all formula of hydrogel was stable in organoleptic, homogeneity and spread test, but the pH value was on 9 which is higher than normal skin pH. Otherwise, the water-based cream was stable in organoleptic, pH and spread test, but in formula 1 showed an emulsion phase separation and formula 3 showed a non-homogeneous arrangement and visible grain after 14 days of storage. Both of the preparation was suitable to applicate cherry leaves extract in antibacterial dosage form with data of skin irritation test.

1. Introduction
Indonesia has many types of plants that are used as traditional medicine [1]. One of that potential plants is cherry (Muntingia calabura L.). Cherry is often found in tropical areas, including Indonesia, Philippines and Mexico. Empirically, Cherry leaves are used as an antipyretic, anti-inflammatory, antibacterial, and also as a natural antiseptic. There are bioactive compounds in cherry leaves that shows the antibacterial activity such as flavonoids, saponins and tannins [2]. Flavonoids are the largest group of natural phenolic compounds and are the polar compounds because theirs several hydroxyl groups can dissolve in polar solvents such as ethanol and methanol. Flavonoids are active compounds that can potential as an antioxidant, anti-inflammatory and antibacterial because they inhibit the activity of disease-causing bacteria [3].

The activity test of the ethanol extract of cherry leaves (Muntingia calabura L.) has been reported on Staphylococcus epidermidis as a bacterium that causes acne. The results at concentrations of 1, 3, 5, and 9 ppm showed that the extract in concentrations of 3, 5 and 9 ppm had an antibacterial activity against Staphylococcus epidermidis, but at a concentration of 1 ppm did not inhibit the activity of the tested bacteria [4]. The antibacterial activity of ethanol extract of cherry leaves showed a positive relationship between the concentration and the diameter of the inhibition area. The higher concentration of the extract, the greater activities of the antibacterial compound contained in the ethanol extract of cherry leaves. It means the extract has a large inhibitory ability to against bacteria [5]. Several types of anti-acne preparations appear in hydrogel and cream preparations. The gel is widely used because it has
several advantages, such as more comfortable and cooler when applied on the skin, more convenient to spread, more adhesive but it does not plug the pores, better to release the active agent, and easier to become dry to form a film layer when we wash off from the skin [6]. Moreover, hydrogels have been extensively studied as an alternative material for antibacterial applications and some types of hydrogels also have an inherent antibacterial property [7]. Oil-in-water cream preparations are also widely used for cosmetics and aesthetics product because they are easier to apply and more comfortable to use on the face. Besides that, they are not sticky and are easier to wash with water than water-in-oil cream preparations [8].

In this research, cherry leaves extracts (Muntingia calabura L.) was formulated in gel and water-based cream preparation in order to compare the physical stability of the dosage form by various test such as organoleptic, pH, homogeneity, spreadability, and emulsion stability test for cream preparations. The results have to followed by skin irritation test to preserve the application on the skin as an antibacterial preparation.

2. Materials dan methods

2.1. Materials

Samples of cherry leaves were collected on Palu City. Materials for the hydrogel include carbomer, methylparaben, propylene glycol, triethanolamine (TEA), and aquadest. Materials for the cream include stearic acid, methylparaben, liquid paraffin, TEA, cetyl alcohol, propylparaben, rose oil, and aquadest.

2.2. Extraction

60 g dried simplicia were weighed, wrapped in filter paper and inserted into the thimble in soxhlet device that has been installed. Then, ethanol 96% were added to each 500 ml round bottom flask. Extraction was processed until the color of the solvent becomes clear or colorless. Then the obtained liquid extracts were evaporated by using rotary evaporator to reach a viscous extracts.

2.3. Formulation of hydrogel and water-based cream

Hydrogel was prepared using carbomer base that dispersed into warm water, allowed until swelling, and homogenized. Then TEA was added and mixed until a gel base was formed (then it called mixture 1). Propylene glycol and methylparaben were diluted using 70% ethanol into the mixture 1 (mixture 2). Then, ethanol extracts of cherry leaves were deliberated to the mixture 2 and filled with aquadest until 60 ml while continuously stirred to produce a stable and homogenous hydrogel.

| Table 1. Hydrogel formula of ethanol extracts Cherry leaves |
|----------------------------------------------------------|
| Ingredients                                              | % w/w |
|----------------------------------------------------------|
| Ethanol extracts of cherry leaves                        | 9     |
| Carborner                                                | 1     |
| TEA                                                      | 3     |
| Propylene glycol                                         | 15    |
| Methylparaben                                            | 0.1   |
| Aquadest                                                 | add 100 |
|                                                        | F1    |
|                                                        | F2    |
|                                                        | F3    |

The water-based cream was prepared by mixed the oil phase including stearic acid, liquid paraffin, cetyl alcohol, and propylparaben that melted at ± 70°C then stirred until homogeneous. Then the ethanol extracts of cherry leaves were dissolved in water phase that included TEA, glycerin and methylparaben that heated at the same temperature as the oil phase. After that, the water phase was added into the oil phase continuously until a cream mass was formed.
Table 2. Water-based formula of ethanol extracts Cherry leaves

| Ingredients                        | % w/w | F1    | F2    | F3    |
|------------------------------------|-------|-------|-------|-------|
| Ethanol extracts of Cherry leaves  | 9     | 9     | 9     |
| Stearic acid                       | 6     | 12    | 18    |
| Liquid paraffin                    | 1.9   | 1.9   | 1.9   |
| Cetyl alcohol                      | 3     | 3     | 3     |
| Propylparaben                      | 0.02  | 0.02  | 0.02  |
| TEA                                | 2     | 3     | 4     |
| Glycerin                           | 15    | 15    | 15    |
| Methylparaben                      | 0.18  | 0.18  | 0.18  |
| Rose oil                           | 0.5   | 0.5   | 0.5   |
| Aquadest                           | add 100 | add 100 | add 100 |

2.4. Physical stability test of hydrogel and water-based cream
The results of evaluation were identified after storage for 1, 7 and 14 days that including an organoleptic, pH, homogeneity, spreadability, and emulsion type test, and also stability test for cream dosage form.

2.5. Data analysis
Descriptive analysis was applied on several test data such as organoleptic, homogeneity, emulsion type test, and emulsion stability test. While statistical analysis was conducted on the data of pH test and spreadability test using One-Way ANOVA and followed by the Post-Hoc test.

3. Results and discussion
Soxhlation was processed for 1 day using 60 g of cherry leaves simplicia in 1000 ml of 96% ethanol as a solvent. The results of liquid extracts were 85% and viscous extracts were 2.58%. The extracts were dark green in color, cherry leaves spesific in odor, and viscous in consistency.

Table 3. The result of phytochemical content test in ethanol extracts of Cherry leaves

| Sample         | Phytochemical Content |
|----------------|-----------------------|
|                | Flavonoid | Saponin | Tannin |
| Ethanol extracts| +         | +       | +      |

3.1. Physical stability test of hydrogel and water-based cream
3.1.1. Organoleptics test. The results of the organoleptics test can be seen in Table 3 that showed all formulas appeared in a yellowish brown gel with smell of cherry leaves. Based on the observed gel consistency, formula 1 produced a thinner consistency than formula 2 and 3. After 14 days storage, all gel preparations were stable organoleptically, which means no change in color, odor and consistency.
Table 4. The result of organoleptic test in hydrogel of cherry leaves ethanol extracts

| Organoleptic Parameters | Storage Time (day) | Formula 1 | Formula 2 | Formula 3 |
|-------------------------|-------------------|-----------|-----------|-----------|
| Color                   | 1                 | Yellowish brown | Yellowish brown | Yellowish brown |
|                         | 7                 | Yellowish brown | Yellowish brown | Yellowish brown |
|                         | 14                | Yellowish brown | Yellowish brown | Yellowish brown |
| Odor                    | 1                 | Cherry leaves | Cherry leaves | Cherry leaves |
|                         | 7                 | Cherry leaves | Cherry leaves | Cherry leaves |
|                         | 14                | Cherry leaves | Cherry leaves | Cherry leaves |
| Consistency             | 1                 | Thinner, transparent | Slightly thick, transparent | Viscous, transparent |
|                         | 7                 | Thinner, transparent | Slightly thick, transparent | Viscous, transparent |
|                         | 14                | Thinner, transparent | Slightly thick, transparent | Viscous, transparent |

Besides the gel, the result of organoleptics test was also observed in water-based cream preparation that showed in Table 4. All cream formulas was stable organoleptically after 14 days storage that represented no change in odor (rose oil) and in consistency (viscous), but there were changes in color from dark green to brownish green after storage at day 14.

Table 5. The result of organoleptic test in water-base cream of Cherry leaves ethanol extracts

| Organoleptic Parameters | Storage Time (day) | Formula 1 | Formula 2 | Formula 3 |
|-------------------------|-------------------|-----------|-----------|-----------|
| Color                   | 1                 | Dark green | Dark green | Dark green |
|                         | 7                 | Dark green | Dark green | Dark green |
|                         | 14                | Brownish green | Brownish green | Brownish green |
| Odor                    | 1                 | Rose oil | Rose oil | Rose oil |
|                         | 7                 | Rose oil | Rose oil | Rose oil |
|                         | 14                | Rose oil | Rose oil | Rose oil |
| Consistency             | 1                 | Viscous | Viscous | Viscous |
|                         | 7                 | Viscous | Viscous | Viscous |
|                         | 14                | Viscous | Viscous | Viscous |

3.1.2. pH test. The results of pH test on all gel formulas showed that the pH was remained stable on pH 9 during 14 days of storage. On the contrary, the pH of the water-based cream formulas presented no change in the values at pH 6 from day 1 to day 14. pH of the cream was more acceptable to apply on the skin because it is in the range of skin pH at 4.5 – 6.5 [9]. The results of statistical analysis with ANOVA on the two preparations were also not significantly different between all formulas (Sig > 0.05). They indicates that storage does not affect changes in pH of the preparation.

3.1.3. Homogeneity test. The results of homogeneity test on the gel which observed for 14 days showed that all preparation had a homogenous arrangement and stable. Meanwhile, the test results on the cream showed that the formula 1 and 2 had no coarse grains, while formula 3 showed the presence of coarse grains after 14 days storage.

3.1.4. Spreadability test. The wide spreading of the 3 gel formulas were different. Formula 1 covered the largest spreading area of all formulas that reached 5.5 cm³. The results of statistical analysis showed
that all formulas were significantly different (Sig < 0.05). It exhibited that there is an effect of variations in carbomer concentration when forming a gel base. The results of further tests with Post-Hoc also showed that each formula was significantly different. Increasing the carbomer concentration reduces spreadability of the gel. Factor that affects the dispersion ability is the strength of gel matrix, which is the stronger the matrix, the lower the dispersion of the gel. The formation of the gel matrix is influenced by the gelling agent, where the higher the concentration, the stronger the gel matrix was formed [10].

While the spreading test results of all cream formulas during the 14 days storage showed good dispersibility at range 4.5 until 5.0 cm³. The results of ANOVA that followed by Post-Hoc conducted the differences between each formula was significant (Sig < 0.05). It means that there is influence of storage on the spreadability of the cream.

From both of the results above, it can be concluded that the types of preparation affected their spreading on the skin. However, the spread range is still included in the good dispersion value of the preparation, which is between 5 – 7 cm² [11].

3.1.5. Emulsion type test. Emulsion type test is carried out by dissolved the cream partially into the water in the test tube then shaken until homogenous. If the cream preparation well penetrates in water, the cream has oil in water (O/W) type, but if it is not mixed well, the preparation has water in oil (W/O) type.

| Table 6. The result of emulsion type test of Cherry leaves water-based cream |
|-----------------------------------------------|
| Emulsion Type Test | Formula 1 | Formula 2 | Formula 3 |
| Dissolved in water | Dissolved in water | Dissolved in water |

Finally, storage stability of the formula 1 by Freeze Thaw method was acceptable on 2 cycle, but in the 3rd cycle occurred phase separation. However, there was no separation in formula 2 and 3. According to Agoes [12], the occurrence of phase separation is related to a decreasing in viscosity of the cream preparations.

4. Conclusions

The ethanol extract of cherry leaves (M. calabura L.) can be formulated into a gel preparation. All formulas showed a stable physical characteristic organoleptically, exposed a homogenous arrangement, and provided good spreadability. As the pH was unsuitable with the skin pH, the gel requires irritation test data for the application on the skin. Meanwhile, the ethanol extract of cherry leaves (M. calabura L.) can also be formulated into water-based cream preparations. All formulas showed stability in organoleptic, pH and spread test. However, phase separation occurred in formula 1 after storage in the 3rd cycle of Freeze Thaw method and instability also exhibited in formula 3 that presented coarse grains on 7 days storage in homogeneity test.

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