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The Effect of Various Concentrations of the Addition of Emulsifier Tween 80 and Span 80 on the Stability of Cream Formulation Ethanolic Extract of Basil Leaves (*Ocimum Americanum L*)

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ABSTRAK

This study aims to determine the effect of the various concentration of the addition of emulsifier tween 80 and span 80 on the stability of cream preparations containing ethanolic extract of basil leaves (Ocimum americanum L) before and after the freeze-thaw cycling test. The formulation of the cream preparation uses 3 of emulsifier concentrations tween 80 and span 80 (1%, 3%, 5%). The evaluation tests carried out were organoleptic, pH, viscosity, spreadability, adhesion, centrifugal test, emulsion type, homogeneity and freeze-thaw cycling. The results of several tests for the three formulations before and after the freeze-thaw cycling test at temperatures (2-8˚C) and (15-30˚C) for 24 hours, for 3 cycles, such as pH, dispersion, adhesive, and viscosity met the requirements for cream formulation. It has pH 5-5.10, dispersion test 5.1-5.8 cm, adhesiveness test 06.05-06.65 seconds and viscosity test increased significantly with a value range of 2000-30000 cPs. Except, for the centrifugal test of formula 1 because of the separation cream phase. This study concludes that the results of statistical tests analyzed using ANOVA showed no significant difference in the addition of emulsifier tween 80 and span 80 in three variations of cream dosage concentrations before and after freeze-thaw cycling for 3 cycles.

Key word:
Basil;
Cream;
Emulsifier;
Span;
Tween.

ABSTRACT

Penelitian ini bertujuan untuk mengetahui pengaruh konsentrasi penambahan emulgator tween 80 dan span 80 terhadap stabilitas sediaan krim ekstrak etanol daun kemangi (Ocimum americanum L) sebelum dan sesudah uji freeze-thaw cycling. Formulasi sediaan krim menggunakan 3 konsentrasi emulgator tween 80 dan span 80 yaitu (1%, 3%, 5%). Uji evaluasi yang dilakukan adalah uji organoleptik, pH, viskositas, daya sebar, daya lekat, uji sentrifugal, tipe emulsi, homogenitas dan freeze-thaw cycling. Hasil pengujian ketiga formulasi sebelum dan sesudah uji freeze-thaw cycling pada suhu (2-8˚C) dan (15-30˚C) selama 24 jam 3 siklus seperti pH, daya sebar, daya lekat, dan viskositas memenuhi persyaratan. Hasil untuk Uji pH 5-5,10, uji daya sebar 5,1-5,8 cm, uji daya lekat 06,05-06,65 detik dan uji viskositas yang meningkat signifikan untuk formula 1, 2 dan 3 dengan kisaran nilai 2000-30000 cPs. Kecuali, uji sentrifugal formula 1 terjadi pemisahan fase krim. Penelitian ini menyimpulkan bahwa hasil uji statistik di analisis menggunkan ANOVA menunjukan tidak adanya perbedaan yang bermakna pada penambahan emulgator tween 80 dan span 80 pada tiga konsentrasi sediaan krim sebelum dan sesudah freeze-thaw cycling selama 3 siklus.

Pendahuluan

One of the plants in Indonesia that has long been used for medicine is basil (Ocimum americanum L.) leaves from the Lamiaceae family. Basil contains compounds such as essential oils, alkaloids, saponins, flavonoids, triterpenoids,

steroids, tannins and phenols (Rimala, M.,2019). Basil contains active compounds in the form of flavonoids with a protein binding mechanism that can interfere with bacterial metabolic processes (Rimala, M.,2019). The use of antibacterial agents that cause acne will be more effective when used topically. The preferred topical preparation is cream. Cream is a semi-solid preparation containing one or more drug ingredients dissolved or dispersed in a suitable base material. The cream has several advantages compared to other preparations, namely easy to spread evenly, practical, easy to clean or wash, the way the work takes place on local tissues, is not sticky, especially the O/A type, is used as a cosmetic, and the ingredients for topical use are not absorbed enough to be toxic (Syaputri,dkk.,2019). Creams are classified into two types, namely oil in water (O/A) and water in oil (W/O) cream types (Anwar,dkk.,2012). In cream preparations, an emulsifier is needed to stabilize substances that cannot be mixed in it (Husein, dkk.,2019). The emulsifier used is tween 80 and span 80. That belongs to the nonionic group because it has activities that are relatively independent of temperature, easier to wash, non-greasy, low irritation level, not affected by pH, stable to weak acids and weak bases and combinations non-ionic and hydrophilic - lipophilic can form highly structured emulsions (Syaputri,dkk.,2019). The emulsifier used in this study was tween 80 and span 80 with a concentration of formula 1 (1%), formula 2 (3%) and formula 3 (5%).

Metode

Tools and materials

Tools

Vacuum rotary evaporator (Buchi), Viskometer (Brookfield), Centrifuge (Orcgon), laboratory glassware, mortar and stamper, water bath, and pH meters.

Materials

70% ethanol, adeps lanae, and aquadest were purchased from Metro Alkes, parafin liquid, tween 80, span 80, nipagin, and nipasol, were purchased from Sarana Abdi Bakti, FeCl3,HCl 2N, cetyl alcohol dragendroff's reagent, chloroform, anhydrous acetic acid, and concentrated sulfuric acid were purchased from Chemistry Mart, basil leaf samples were taken from Tanjungsari Village, Kec. Banjaranyar, Kab. Ciamis.

Methods

1. Basil leaves Extraction

Maceration of basil leaves is done by soaking 900 grams of basil leaves in a glass jar, adding 18 L of 70% ethanol, soaking for 24 hours and occasionally needing to stir (Kumalasari, dkk.,2020) the filtrate from the maceration was concentrated using a rotary evaporator (Kumalasari, dkk.,2020).

2. Phytochemical Screening

A. Flavonoid test

Basil leaf extract as much as 10 mg plus 5 mL of ethanol and a few drops of $FeCl_3$ until the colour changes. The content of flavonoids is indicated by a colour change to blue, purple, green, red or black. If up to 20 drops of the addition of FeCl3 there is no colour change, then the extract is declared negative for containing flavonoids (Husein, dkk.,2019).

B. Alkaloid Test

2 mL of the test solution was evaporated over a porcelain cup to obtain a residue. The residue was then dissolved with 5 mL of 2N HCl. After cooling, the solution is filtered. The solution obtained was divided into 3 test tubes. The first tube serves as a control. The second tube was added with 3 drops of Dragendroff's reagent and the third tube was added with 3 drops of Mayer's reagent (through the tube wall). The formation of an orange precipitate in the second tube and a yellow precipitate in the third tube indicates the presence of alkaloids (Yuda,dkk.,2017).

C. Tannin test

As much as 0.5 grams of basil leaf extract was boiled in 20 mL of distilled water in a test tube. Filter and add few drops of 0.1% FeCl3 until it changes colour. Positive results containing tannins are indicated by the appearance of a green colour brownish or blue-black colour (Husein, dkk.,2019).

D. Saponin Test

0.5 grams extract was added with 5 ml of distilled water, shaken vigorously. A positive test for the presence of saponins in the solution is indicated by the formation of foam (Husein, dkk.,2019).

E. steroids and triterpenoids Test

The extract was dissolved in chloroform then added with Liebermann-Bouchard reagent (anhydrous acetic acid-H2SO4) showed positive results with a colour change to brownish red for steroids and brown-purple for triterpenoids. The reaction of triterpenoids with Liebermann's reagent produces a red purple colour while steroids give a green-blue

colour (Chandra, dkk.,2019).

F. Anthraquinone Test

A total of 50 mg of extract plus 10 mL of water then heated for 5 minutes and filtered. A total of 3 mL of the solution was put into 2 test tubes, tube 1 was added a few drops of 1 N NaOH solution, if positive, a red solution was formed and tube 2 was used as a control (Yuda,dkk.,2017).

3. Cream Formulation

The oil phase is made by melting span 80, liquid paraffin, nipasol, cetyl alcohol and adeps lanae on a water bath with a temperature of 70ºC, then the aqueous phase is made by dissolving nipagin, tween 80, basil leaf extract on a water bath and input the oil phase into aqueous phase while stirring in a hot mortar until a stable corpus is formed (Puspita,dkk.,2020).

Tabel 1. Basil leaf extract cream formulation

Bahan	F1 (%b/b)	F2 (% b/b)	F3 (% b/b)	Fungsi
Ekstrak daun kemangi	4	4	4	Zat Aktif
Tween 80	1	3	5	Emulgator
Span 80	1	3	5	Emulgator
Parafin liquid	5	5	5	*Emolient*
Adeps lanae	7,5	7,5	7,5	Basis Krim
Setil alkohol	8	8	8	Pengental
Nipagin	0,2	0,2	0,2	Pengawet
Nipasol	0,02	0,02	0,02	Pengawet
Aquadest ad	100	100	100	Pelarut

4. Freeze-thaw cycling

The cream samples of each formula were stored in the refrigerator at cold temperatures (2-8˚C) for 24 hours, then placed at room temperature (15-30˚C) for 24 hours. The test was carried out for 3 cycles (Wardani,dkk.,2020).

5. Evaluation Test

A. Organoleptic

The cream samples of each formula were observed with the naked eye for the colour, texture and odor of the product (Wardani,dkk.,2020).

B. Homogeneity Test

The preparation was observed subjectively by applying a little cream on the slide and observing the composition of the particles formed. Replication was carried out 3 times (Wardani,dkk.,2020).

C. Viscosity

Cream samples were measured with a Brookfield VP1000 viscometer using spindle number 7 with a speed set at 5 rpm, 10 rpm, 20 rpm and 50 rpm, respectively. The replication was carried out 3 times (Wardani,dkk.,2020).

D. pH test

The preparation was weighed as much as 5 g and dissolved in 45 ml of distilled water. The pH meter is immersed in the diluted sample. The indicated pH value is recorded.

Replication was carried out 3 times (Puspita,dkk.,2020).

E. Spreadability test

A total of 500 mg of cream was placed on a round glass and then covered with a round glass. A load weighing 50 g is placed on it and allowed to stand for 1 minute, recorded and the diameter of the spread is calculated. Replication was carried out 3 times (Puspita,dkk.,2020).

F. Adhesion test

A total of 0.25 grams of cream was smeared on a glass plate, the two plates were affixed until the plates were fused, pressed with a load of 50 g for 5 minutes. The glass plates are attached to the adhesive test equipment and removed with a load of 80 g, then the time when the two plates are removed is recorded. Replication was carried out 3 times (Puspita,dkk.,2020).

G. Phase separation test

10 g of cream sample was placed in a centrifugation tube and centrifuged at 4000 rpm for 30 minutes, then phase separation occurred. Replication was carried out 3 times (Puspita,dkk.,2020).

H. Emulsion type test

The cream that has been made is put into a beaker and then a few drops of methylene blue solution are added. If the blue colour is immediately dispersed throughout the emulsion, then the emulsion type is O/W, otherwise, if the blue colour is not completely dispersed then the emulsion type is W/O. Replication was carried out 3 times (Warnida,dkk.,(2017).

Results And Discussion

Extraction

The results of the extraction process obtained a thick extract of basil leaves as much as 62.88 grams with a % yield value of 6.98%. This yield value is low compared to previous studies which stated that the average yield of basil leaf extract ranged from 24.06 to 33.67% (Rofiatul,dkk.,2015). The low yield value obtained is due to the maceration process that is not optimal, the sample size is not appropriate because the smaller the sample size, the larger the area between the solid and the liquid so that precise contact is possible, and the selection of the solvent is not appropriate so that the extract obtained is small. and the compounds obtained are also small (Sharon,dkk.,2013).

Phytochemical Screening

The results of the phytochemical screening test, it can be stated that the thick ethanolic extract of basil leaves is positive for flavonoids, saponins, tannins, and steroids but does not contain alkaloids, anthraquinones and triterpenoids, can be seen in table 2 (Vinnata,dkk.,2018).

Table 2. Phytochemical screening results of basil leaf ekstract

No	Testing	Results
1.	Flavonoid Test	(+) Positive
		(+) Positive
2.	Saponin Test	(+) Positive
3.	Alkaloid Test	(-) Negative
4.	Tanin Test	(+) Positive
5.	Antrakuinon Test	(-) Negative
6.	Steroid Test	(+) Positive
7.	Triterpenoid Test	(-) Negative

A. Organoleptic Cream

Preparations made with 3 variations of formula concentration before and after freeze-thaw cycling resulted in a light green, smooth cream preparation with a characteristic basil odor.

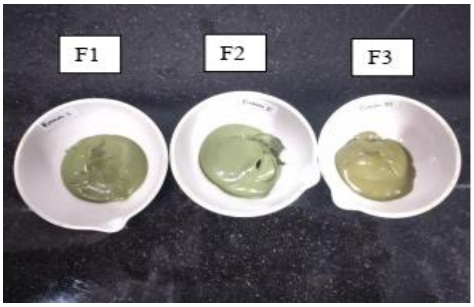


Figure 1. Organoleptic basil leaf extract creams

B. Homogeneity

The results of homogeneity before and after freeze-thaw cycling resulted in a cream that was homogeneous, smooth, and no particles grain obtained.

C. Viscosity

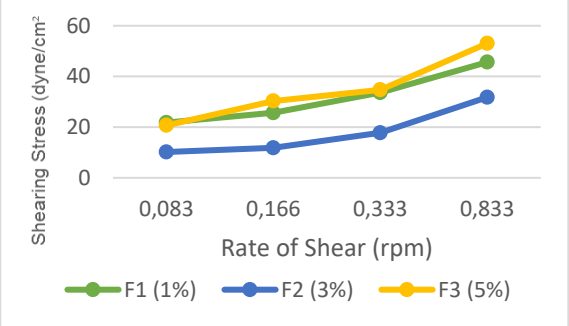


Figure 2. Viscosity test results before freeze thaw cycling

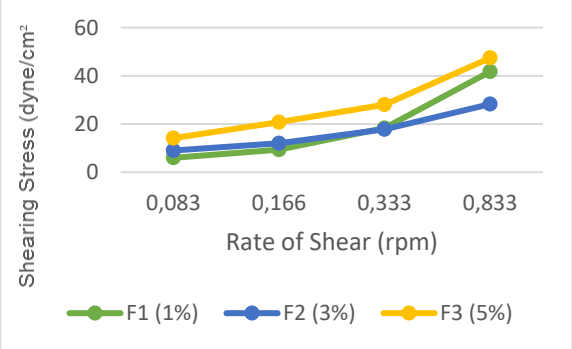
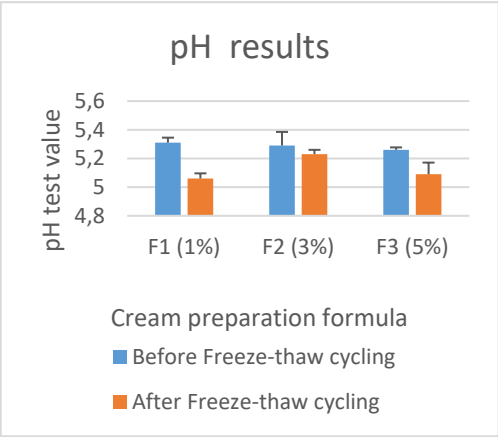


Figure 3. Viscosity test results after freeze thaw cycling

The viscosity test aims to see the flow type of a cream preparation because a good cream preparation must have pseudoplastic flow properties. The results of the viscosity test of the basil leaf ethanol extract cream before and after Freeze thaw cycling showed that the three formulas had a viscosity value that was in accordance with the requirements of the cream preparation, namely 2000-50,000 (Warnida,dkk.,(2017). The results of the rheological graph shown in Figures 2 and 3 produced before and after the freeze thaw cycling test stated that the resulting cream was included in the thixotropic type of Pseudoplastic flow because it was characterized by a hysteresis loop between the ascending and descending curves. This loop area indicates the time required for a structure to return to its original state after the force is removed (Caesaron,dkk.,2015).

D. pH test

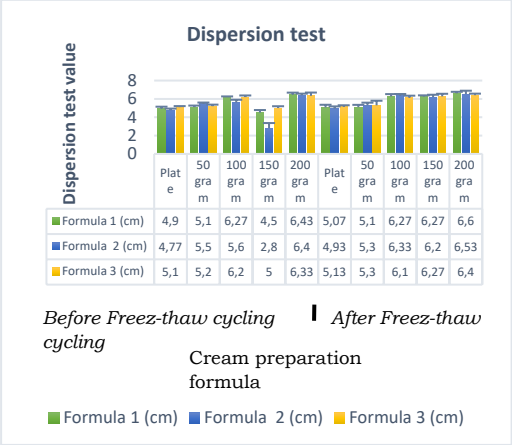


*p > 0.05

Figure 4. pH test results before and after freeze thaw cycling

From the results of the pH test on the cream preparation, it was found that the higher the emulsifier used, the lower the resulting pH value and also a decrease in the pH value after the freeze-thaw cycling test was carried out, but it was still within the required pH range for the cream, as shown in Figure 4. The cream preparation must be in accordance with a skin pH ranging from 4.5 to 6.5. Preparations with a pH value that is more acidic than the pH of the skin allow irritation during application, while if the pH of the preparation is more alkaline than the normal pH of the skin, it can cause the skin to become dry during application (Ainaro,dkk.,2015). The results of the ANOVA test stated that there was no significant difference between the three formulas.

E. Spreadability test



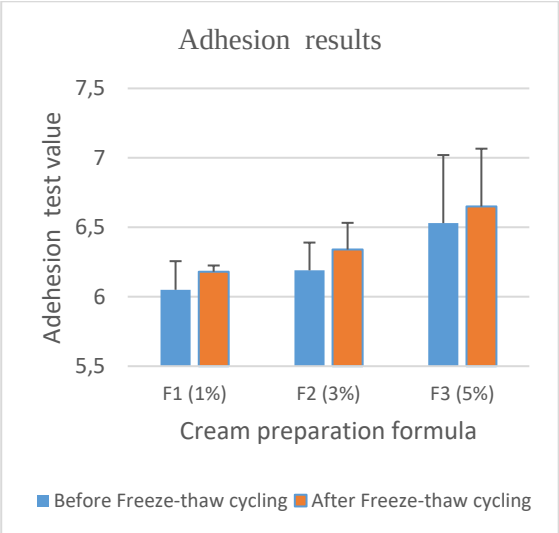
*p > 0.05

Figure 5. Spreadability test results before and after freeze thaw cycling

The spreadability test of cream preparations vis carried out to determine the amount of power required for the cream to spread on the skin or to determine the ability to spread cream preparations when applied to the skin (Mardikasari,dkk.,2020). Good dispersion of cream preparations for topical use ranges from 5-7cm in diameter (Mardikasari,dkk.,2020). The results of the increased dispersion test for each formula after the freeze-thaw cycling test are shown in Figure 5 and the higher the emulsifier, the lower the dispersion value but still within the required range of dispersion power of the cream preparation. The results of the ANOVA test stated that there was no significant difference between the three formulas.

F. Adhesion test

Adherence test is carried out to test how long this cream will stick to the skin when applied because it will affect how long and how much drug or active substance will be absorbed by the skin. The requirement for good adhesion of cream preparations is not less than 4 second (Mardikasari,dkk.,2020). In this test, the results showed that all formulas showed good adhesion values before and after the freeze-thaw cycling test as shown in Figure 6. The higher the concentration of emulsifier used, the higher the adhesion value produced but still within the range of adhesion requirements. . The results of the ANOVA test stated that there was no significant difference between the three formulas.



*p > 0.05

Figure 6. adhesion test results before and after freeze thaw cycling.

G. Phase separation test

From the test results before and after the freeze-thaw cycling test, it was found that formula I was separated into 2 parts with a creaming height of 1 cm so that the creaming index was 75%. The greater the creaming index produced, the less creaming is

formed. Meanwhile, for the results of formulas 2 & 3 there was no phase separation/creaming so it can be stated that formulas 2 & 3 were stable in 1 year storage (Yuda,dkk.,2017).

H. Emulsion type test

The emulsion type test was carried out to determine whether the cream preparations were classified as oil-in-water or water-in-oil emulsion types by dripping the cream with methylene blue. The results of observations before and after freeze-thaw cycling showed that the three cream formulas were classified as oil in water (w/a) as seen from the methylene blue which was completely dispersed in the cream preparation (Anwar,dkk.,2012).

Conclusion

The physical and chemical characteristics of cream preparations containing basil leaf extract (Ocimum americanum L) with different concentrations of emulsifier tween 80 and span 80 in formula 1 (1%), formula 2 (3%) and formula 3 (5%) showed that formula 3 is the most optimal formula seen from all the results of testing the physical properties and physical stability of the cream, especially in the adhesion test it can be seen that formula 3 produces the longest adhesion time and physical stability compared to formula 2 and formula 1.

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