

Potency and Stability Emulgel of Ethanol Extract Strawberry Leaf (*Fragaria x ananassa var Duchesne*) as a Sunscreen

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ABSTRACT

Strawberry leaf extract (*Fragaria x ananassa var Duchesne*) reported having good sunscreen activity. However, a suitable dosage formulation is necessary for the next stage. Therefore, this study aimed to formulate emulgel from the ethanol extract of strawberry leaves using physical properties test, stability tests, and SPF value tests. The strawberry leaves were extracted with the reflux method with 96% ethanol. The extract was analyzed for chemical content by TLC. The emulgel formulated with 0.25%, 0.50%, and 1% extract. The emulgel was tested for physical properties, stability, and in vitro and in vivo sunscreen activity. The results showed that the yield of ethanol extract of strawberry leaves was 20.5%, and the TLC test showed positive content of flavonoids and tannins. The emulgel formula had the best effectiveness as a barrier was formula with an extract concentration of 1%. The 1% ethanol extract concentration of strawberry leaves and emulgel resulted in an SPF value of 59.94 ± 0.260 .

Keywords: emulgel; strawberry leaves; sunscreen

INTRODUCTION

Strawberry leaf extract has antioxidant activity with phenolic compounds, polyphenols, gallate, vanilla, 3-O-hydroxybenzoate, quercetin-3-O-rutinoside, quercetin-3-O-glucopyranoside, catechins, caffeic acid, ellagic acid, quercetin, myricetin and kaempferol¹. From these ingredients, this extract has a strong potential for making sunscreens. Previous research has shown that the ethanol extract of strawberry leaves has antioxidant activity with an IC50 value of 363.551 ppm and activity as a sunscreen with an SPF value of 20.090 at a concentration of 175 ppm². This study formulated an emulgel sunscreen using the active substance of the ethanol extract of strawberry leaves with different concentrations. Previous research has carried out the ethanol cream formulation of strawberry leaves with a concentration of 0.50%, giving an ultra SPF value of 41.20³, and 1,00 % giving an ultra SPF value of 52.9⁴, Emulgel formula with 1% cinnamon bark extract⁵, 5 and 10 % guava leaves extract⁶ as the active ingredient showed stable emulgel results. The novelty of this research is the choice of emulgel preparation. These preparations have the advantage of good drug conductivity by providing faster drug release than ointments and creams⁷.

Sunscreen activity in vitro test using UV-Vis spectrophotometry. The sunscreen activity test was carried out by determining the SPF value of the preparation and then testing its safety on the skin of several volunteers. The research objective was to formulate an emulgel sunscreen with ethanol extract from strawberry leaves using physical quality, stability, and SPF value tests.

METHODOLOGY

Materials

The materials used were strawberry leaves (*Fragaria x ananassa var duchesne*) from Tawangmangu, Karanganyar, Central Java, 96% ethanol (Rofa Laboratorium Center), methylene blue (Merck), liquid paraffin (T&T Chemical), tween 80 (CV. Kimia Jaya Labora), span 80 (CV. Kimia Jaya Labora), methylparaben (CV. Kimia Jaya Labora), propylparaben (CV. Kimia Jaya Labora), propylene Glycol (CV. Kimia Jaya Labora), carbopol 940 (Kimia Jaya Labora), triethanolamine (Petronas Chemical) ethanol pro analysis (PT. Smart Lab) and distilled water.

Utilized instruments

The instruments used in this research are UV-VIS spectrophotometers (Shimadzu UV-

1800), viscometer (Rion VT 047), and pH-meter (Oakion).

METHOD

Plant determination was carried out at the Center for Research and Development of Traditional Medicinal Plants (B2P2TOT) Tawangmangu. The strawberry leaves in the study came from a strawberry plantation in Tawangmangu, Karanganyar, Central Java, in August 2020. Strawberry leaves were cleaned, mashed, and sieved (40mesh), strawberry leaves powder was weighed at 100 grams and extracted by reflux for 3 hours with 600 ml of 96% ethanol, and the solvent was removed by vacuum rotary evaporator. Identification of flavonoids and tannins in extracts using Thin Layer Chromatography (TLC). TLC of flavonoids, methanol eluent: water (9,5: 0,5), In this analysis, quercetin was used as a comparison compound meanwhile TLC of tannins, ethyl acetate eluent: methanol: water (100: 13,5: 10), In this analysis, gallic acid was used as a comparison compound ⁸.

Formulation Emulgel as A Sunscreen

The strawberry leaf extract emulgel formulation was prepared using variations in the extract concentration, namely 0.25%, 0.50%, and 1%. The selection of concentration variations was based on previous research, namely making emulsion preparations of ethanol extract of strawberry leaves with a concentration of 0.50%, resulting in an SPF value of 41.20 ± 0.072 ³.

The oil phase was prepared by mixing liquid paraffin, span 80, and nipasol at 70°C. The aqueous phase was prepared by mixing tween 80, propylene glycol, and nipagin with water at 70°C. The oil phase was added to the water phase at a temperature of 70°C while continuously stirring with a stirrer until an emulsion was formed. The gel was made by dispersing Carbopol with little by little hot water at a temperature of 80°C and ground until a gel base was formed.

Emulgel Physical Properties Test

Physical Properties tests carried out included organoleptic test (shape, color, and odor), pH test, viscosity test, adhesion test, dispersion test, homogeneity test, stability test using the cycling test method, emulsion type test using the coloring method, and centrifugation

test for 30 minutes at a speed of 5000 rpm. In addition, statistical analysis was performed using the SPSS (Statistical Package for the Social Science) program with the ANOVA (Analysis of Variance) method.

SPF Value of Sunscreen Emulgel

Determining the SPF value using a 20% ethanol-soluble emulgel concentration in each formula, read the absorption with a UV-Vis spectrophotometer at a wavelength of 290-320 nm with intervals of every 5 nm. Then, the SPF absorption value is calculated using the Mansur formula⁹.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Information: CF : Correction factor; I : intensity; Abs : absorbance of sampel; Nilai EE x I : constant value

Irritation Test

The irritation test was carried out by using an open patch test on the skin of the volunteers. There were 12 volunteers who were used as panels in the irritation test, with the following criteria: healthy men or women aged over 18 years, physically and mentally healthy, have no history of allergic disease, and are willing to volunteer for the irritation test ¹⁰. A positive irritant reaction is characterized by the presence of redness, itching, or swelling of the treated hand skin ¹¹. The results of erythema and edema were calculated for the irritation index.

Index irritation =

$$\frac{(Score\ eritema\ 24 + 48 + 72) + (Score\ edema\ 24 + 48 + 72)}{number\ of\ volunteers}$$

RESULT AND DISCUSSIONS

Determination at B2P2TOT Tawangmangu showed that the leaves of the plants used were strawberry leaves with the species name *Fragaria x ananassa* (Duchesne ex Weston). The strawberry leaf extract obtained by the reflux method was brownish-green and thick, with a moisture content of 6.2%. The yield fulfills the moisture content requirement of less than 10%. The yield of the extract obtained was 20.82%. The yield of the extract was lower than in previous studies using the maceration method, namely 23% ³. The difference in results is presumably because the extraction time carried out in maceration is longer than that of

Table I. Formulation emulgel of strawberry leaf extract

Ingredients	Concentration(%)			
	Formula 1	Formula 2	Formula 3	Formula 4
Strawberry leaf extract	0.25	0.50	1	-
Paraffin liquid	5	5	5	5
Tween 80	3,5	3,5	3,5	3,5
Span 80	1,5	1,5	1,5	1,5
Methyl paraben	0,18	0,18	0,18	0,18
Propyl paraben	0,2	0,2	0,2	0,2
Propylene Glycol	10	10	10	10
Carbopol	1	1	1	1
TEA	<i>qs</i>	<i>Qs</i>	<i>qs</i>	<i>qs</i>
Aquadest <i>ad</i>	100	100	100	100

Table II. Value of EE x I in wavelength 290-320 nm

Wavelength (λ nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1

reflux. So that the possibility of the compound withdrawal process is maximized, but in terms of time and the amount of solvent used, reflux has better efficiency. The results of the qualitative test for compounds in the ethanol extract of strawberry leaves showed that they were positive for containing flavonoids and tannins. The flavonoid TLC test showed that the Rf value of extract was 0.68 and the standard Rf of quercetin was 0.68, while TLC for tannins obtained the Rf value of extract 0.6 and standard Rf of gallic acid of 0.6. This is in accordance with research by Putri ³.

Physical properties test

The organoleptic evaluation results of the four formulas have a thick texture as expected because the use of a gel base, namely carbopol 940, is 1%. The color of the preparation gets darker with the increasing concentration of the extract. The pH measurement ranges from 5.5 to 5.9, but from each formula, there is no significant difference based on statistical tests. This means that the difference in extract concentration does not affect the pH of the preparation. Previous studies have had a pH

value of 7.9-8.1 in cream with ethanol extract from strawberry leaves ³. This means that the emulgel is more acidic than the cream. The influencing factors were the dosage form, the extract, and the concentration used. These results indicate that the pH of the emulgel is better than the cream because it has a value that is in the range of requirements according to SNI, namely 4.5-7. Differences influence the viscosity of formulas 1, 2, and 3 in the extracts' concentration.

The greater the extract concentration, the lower the viscosity of the emulgel preparation, very thick and sticky extract is the reason for the difference in viscosity of the emulgel. The viscosity of the three formulas is still within the expected range of 40-400 dPas ¹². There are no special requirements for the adhesion of semi-solid preparations, but it is preferable that the adhesion of semi-solid preparations is more than 1 second ¹³. The adhesion test results are as expected because they range from 3-to 4 seconds. Emulgel adhesion did not significantly differ from formulas 1, 2, and 3, which means that the extract concentration did not affect the adhesion of the preparations. Observation of the

Table III. Physical quality test of emulgel strawberry leaf extract

Sample	Organoleptic	pH	Viscosity	Adhesion	Dispersibility (cm)			
					0g	50g	100g	150g
F1	Light green	5,87±0,01	111,67±2,89	3,80±0,01	4,85	5,62	6,05	6,52
F2	Slightly dark green	5,94±0,02	96,67±2,89	3,66±0,01	4,82	5,45	5,92	6,37
F3	Dark green	5,92±0,01	81,67±2,89	3,55±0,01	4,30	5,02	5,55	5,90
Base	White	5,51±0,01	201,67±2,89	3,92±0,01	4,25	5,20	5,50	5,57

emulgel type was carried out by using the staining method. The emulgel from each formula is an oil-in-water or M / A emulsion type, and as expected.

The results of the spreadability of each formula were not too much different. All of them were in a good range and, as expected, between 4-7 cm. This result is better than previous studies on cream preparations. The ethanol extract cream of strawberry leaves has a dispersion power that does not meet the requirements, which ranges from 3-4 cm³. The results of the homogeneity examination are as expected. Namely, the active substance is evenly distributed and homogeneous in the preparation, characterized by uniform color, smooth texture, and the presence or absence of particles in the emulgel preparation. Centrifugation testing aims to determine the stability between phases of emulgel preparations. The centrifugation speed is 5000 rpm for 30 minutes. This treatment was the same as the treatment for one year of gravity¹⁴. The results of the centrifugation test showed that the emulsion produced in the manufacture of emulgel has good stability because it does not separate.

The next test is the stability test using the cycling test method. To see the changes that occur, the test is carried out in 6 cycles¹⁵. The results of observations before and after the cycling test on viscosity showed that there was a decrease in the viscosity of each formula. The decrease in viscosity is proportional to the decrease in pH of the preparation because the viscosity of carbopol as a gelling agent will decrease with decreasing pH. However, the decrease in viscosity is still within the expected range. As a result of pH measurement, there was an insignificant decrease based on statistical tests. The decrease in pH occurs because the ingredients in the formulation can release

hydrogen ions which will increase the acidity⁵. Based on these results, the ethanol extract of strawberry leaves emulgel has good stability and is as expected despite extreme temperature changes.

SPF Value

Determination of SPF using a UV-Vis spectrophotometer at a wavelength of 290-320 nm with 5 nm intervals. These wavelengths represent the UV-B wavelengths (290-320 nm).

Formulas 1, 2, and 3 use a variation of the ethanol extract of strawberry leaves as much as 0.25%, 0.50%, and 1%. The variation in the extract concentration resulted in an SPF of 34.88 ± 0.094; 44.14 ± 0.142; and 59.94 ± 0.60. The SPF value obtained indicates that the greater the extract concentration, the greater the absorption on spectrophotometry, so the SPF value is greater. The SPF results in formulas 1, 2, and 3 are included in the ultra protection category and are in accordance with the expectations of this study. In previous research, the ethanol cream formulation of strawberry leaves with a concentration of 0.50% gave an ultra SPF value of 41.20 ± 0.072 [3]. The results of this study showed that the strawberry leaf extract emulgel had a better SPF value, namely with a concentration of 0.50%, resulting in an SPF value of 44.14 ± 0.142 and with a concentration of 1%, yielding an SPF value of 59.94 ± 0.260. These differences may be influenced by differences in the extraction method used and the dosage form formulated. The reflux extraction method is carried out by heating. The higher the extraction temperature, the easier the penetration of the solvent into the material so that more samples are extracted¹⁶. This can increase the SPF value of the extract.

Strawberry leaf extract has sunscreen activity due to the presence of an aromatic

Table IV. Cycling test of viscosity and pH emulgel of ethanol extract strawberry leaf

Formula	Viscosity		pH	
	Before	After	Before	After
Formula I	111,67 ± 2,89	101,67±2,89	5,87 ± 0,01	5,78±0,02
Formula II	96,67 ± 2,89	86,67±2,89	5,94 ± 0,02	5,75±0,02
Formula III	81,67 ± 2,89	73,33±2,89	5,92 ± 0,01	5,81±0,02
Base	201,67 ± 2,89	191,67±2,89	5,51 ± 0,01	5,37±0,02

Table V. Result SPF value emulgel of ethanol extract strawberry leaf

Formula	SPF Value	Keterangan
Formula I	34,88±0,094	Ultra
Formula II	44,14±0,142	Ultra
Formula III	59,94±0,260	Ultra
Base	2,78±0,204	Minimal
Control positive	30	Ultra
Extract 0,25%	39,86±0,384	Ultra
Extract 0,5%	51,32±0,586	Ultra
Extract 1 %	68,75±0,840	Ultra

Information : Formula I : emulgel with 0,25% strawberry leaf extract ; Formula II : emulgel with 0,5% strawberry leaf extract; Formula III : emulgel with 1 % strawberry leaf extract; Base : emulgel without strawberry leaf extract (control negative); Control positive : gel sunscreen wardah; Extract : ethanol extract of strawberry leaf

benzene ring in flavonoid compounds. These compounds are powerful antioxidants that can bind metal ions and reduce skin damage¹⁷. The emulgel base has an SPF value of 2.78 ± 0.204 , which means that the base produces very little UV absorbance. The SPF results of the ethanol extract of strawberry leaves with a concentration of 0.25%; 0.5%; and 1% were 39.86 ± 0.384 , respectively; 51.32 ± 0.586 ; and 68.75 ± 0.840 . The SPF value of the extract is higher than that of emulgel preparations. Factors that can influence it include the use of additives, combinations, and effects of carrier component interactions such as emulsifiers used in formulations, the addition of active ingredients, and the pH of preparations¹⁸. The ethanol extract of strawberry fruit at a concentration of 200 µg / ml yielded an SPF value of 20¹⁹. Basically, strawberry fruit has a better SPF value than strawberry leaves, but in this study, the leaves were used because they were considered cheaper because they used parts that were rarely used but had good potential. The positive control in this study was Wardah gel SPF 30 PA ++. Based on the research, the results show that the strawberry leaf extract has excellent potential to be redeveloped, namely an analysis of the effectiveness of

strawberry leaves in addition to being used as a sunscreen.

Result of Irritation Test

The irritation test is carried out to prevent any side effects from the emulgel preparation. However, the value of erythema and edema in human skin from 24 to 72 hours showed no positive reactions in 12 volunteers. Therefore, the result of the irritation index is 0, which means that the preparation does not irritate human skin as expected.

CONCLUSION

Strawberry leaf extract can be formulated in an emulgel preparation with good physical quality and stability and has a sunscreen activity with a 59.94 SPF value at 1%.

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