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Original Research Article

THE FORMULATION OF SUNSCREEN CREAM FROM TELANG FLOWER EXTRACT (CLITOREATERNATEA) AND IN VITRO TESTING OF SUN PROTECTION FACTOR (SPF) VALUE

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ABSTRACT

Introduction. Telang flower contains chemical compounds such as flavonoids that have the pharmacological potential as an antioxidant. The flavonoid compounds in plants are assumed to have antioxidants and the ability to protect the skin from UV rays. **Method**. This research method used a descriptive-analytic approach. The sampling of telang flowers was conducted in the greenhouse garden of the Biology Study Program, Faculty of Science and Technology, PGRI Adi Buana University, Surabaya. **Result&Analysis**.The research stages include the process of extracting flowers by maceration method, the process of microemulsion system from telang flower extract, microemulsions characterization, SPF values testing on microemulsion of telang flower extract and statistical data analysis. **Discussion**. Based on the table of multiple comparison test results in formulation 0 with hypotheses 1, 2 and 3 having a p-value of <0.05, it can be interpreted that there are differences in each formulation. However, there is no difference between formulation 1 and hypothesis 4 which has a p-value of> 0.05.

Keywords: Sunscreen Cream, Telang Flower, In Vitro, Sun Protection Factor.

INTRODUCTION

Sunlight is a gift of nature that has many benefits and has an a important role for the living creatures on earth. Sunlight is one of the main important factors on living things, but it can also have a negative impact on health, especially for skin health. The harmful effects of UV radiation on skin can be divided into 2, called acute effects such as sunburn or erythema, phototoxic reactions, photoallergies and photosensitivity and chronic effects, namely photoaging, skin cancer and immunosuppression (Damayanti, Meylina and Rusli, 2017).

One way to protect the skin from sun exposure is to use sunscreen (Shanbhag et al., 2019). Currently, an in vitro method has been developed to assess the activity of a sunscreen. This method is based on the absorption value of sunscreen determined by spectrophotometric analysis (Adawiyah, 2019).

There are several classes of antioxidant active elements such as cinnamates, flavonoids, tannins, quinones, and others that have been investigated to have the ability to protect the skin from UV rays(Pratiwi and Husni, 2017). One of them is flavonoid components as antioxidants which have been proven with the research results by Sami (2017), that flavonoids also have potential as sunscreens because of the presence of chromophore groups that generally give the color to plants.

Telang flower (Clitoriaternatea), often referred as butterfly pea, is a distinctive flower with a single purple petal. It is known that telang flower is not only an ornamental plant, but it is also known traditionally as a medicinal plant. According to Al-Snafi (2016)and Cahyaningsih (2019), telang flower contains chemical mixtures such as flavonoids, that has the pharmacological potential as an antioxidant. The flavonoid elements in plants is assumed to have antioxidant and the ability to protect the skin from UV rays.

In this research, the ethanol extract of telang flower will be formulated into a cream with various dosage variants. The physical properties of telang flower cream will be evaluated based on the effect of its concentration that can provide a sunscreen effect through an in vitro test of sun protection factor (SPF) value.

METHOD AND ANALYSIS

This research method used descriptive-analytic approach. The sampling and determination of telang flower was conducted in greenhouse garden of Biology Study Program, Faculty of Science and Technology, PGRI Adi Buana University, Surabaya. The sample was measured its length diameter, and the determination test was conducted at UPT Plant Conservation Center LIPI Purwodadi which aims to determine whether the sample species is Clitoriaternatea or not. The results obtained indicate that the plant used in this research is a type of telang flower. This research micro emulsions characterization, SPF values testing on microemulsion of telang flower extract and statistical data analysis.

RESULTS

1. The Treatment and Extraction of Telang flower (Clitoriaternatea)

The samples obtained were dried for approximately four days until the water in plant was reduced for approximately three days in the sun.After the sample is dried, then the dried telang flower is weighed using a digital scale before refining it. The refined samples can be stored in a dry place for the next process, called maceration.

This research using a solvent with 96% ethanol since the ethanol solvent has polar properties which means it can bind many active chemical compounds in plants. The first process weighed 200 grams of telang flower powder which would be extracted using maceration method with 96% ethanol as solvent. The sample was placed in a bottle then added 600 mL of ethanol and allowed let it stand for 24 hours. After that, it filtered using Whatman filter paper no. 42. The filtrate was collected and the residue was macerated with 3 replications until the filtrate was clear. The ethanol extract

filtrate was evaporated using a vacuum rotary evaporator at 50° C temperature to separate its solvent and extract (Tristantini, Ismawati and Jonathan, 2016). and it concentrated using an oven at 55°C for 1 week and weighed the extract obtained.The raw extract obtained was calculated using this formula:

$$\% \text{ yield} = \frac{Total\ extract\ weight}{Total\ sample\ weight} X\ 100\%$$

The results from the extraction of telang flower Clitoriaternatea with a total initial weight of 150 grams of simplicial obtained a concentrated extract weight of 23.47 grams with the total weight of yield was 15.67%. The yield obtained is influenced by the ingredients and the addition of solvents. The more solvents added in extraction process, the bigger the mixing between solvents and simplicial and can be potentially maximizing the extracts result (Handayani et al, 2016). The telang flower extract (Clitoriaternatea) can be seen in Figure 1.

Figure 1. Concentrated extract of telang flower (Clitoriaternatea)

2. Phytochemical Screening Test

2.1 Flavonoid and Tanin Test

The concentrated extract of telang flower was put in a test tube, then dissolved in 1-2 mL of 96% ethanol. After that, the 0.5 g of magnesium (Mg) powder and 4-5 drops of concentrated HCl were added. The red or orange colored solvent indicates the flavonoids(Mojab *et al.*, 2003). The 0.2 grams of concentratedtelangflowerextract was dissolved in 5 mL of 1% FeCL and homogenized, when a blackish brown color apprears, it indicates a positive extract of tannin (Halimah, 2010).

The formation of a blackish green color in the sample after adding 1% FeCl3 to form a complex compounddue to coordination covalent bonds between ions or metals with non-metal atoms (Hermawati, Suhartana and Taslimah, 2016), between tannins and FeCl3 due to the presence of Fe3+ ions as the central atom and tannins have O atoms which have lone pairs of electrons that can coordinate to central atom as the ligand.

Tannins and flavonoids are compounds that have benefits as sunscreens. The activity of condensed tannins has potential as an antioxidant and can protect the skin from damage caused by ultraviolet (UV) radiation. The results of phytochemical tests for flavonoid compounds and tannins from telang flower (Clitoriaternatea) extract that can be seen in Figures 1 and 2.

Figure1

Figure2

Figure 1 The results of phytochemical screening of flavonoid compounds and **Figure 2** The results of phytochemical screening of tannin compounds

2.2 The Microemulsion of Peel Flower Extract

The microemulsion is formed from water phase and oil phase that made separately. The aqueous phase contains tween 80 and aquades, the two materials are heated on a hotplate to a temperature of 50°C then stirred using a magnetic stirrer until the homogeneously is mixed. After that, the oil phase containing span 80, PEG 400 and VCO was homogenized using a magnetic stirrer, the pineapple peel extract was added little by little. Then, water phase and oil phase were mixed, stirred with a magnetic stirrer at 50°C at 1000 rpm speed for ± 30 minutes. After that, a clear and transparent microemulsion was formed which was stable and allowed to stand for 24 hours.

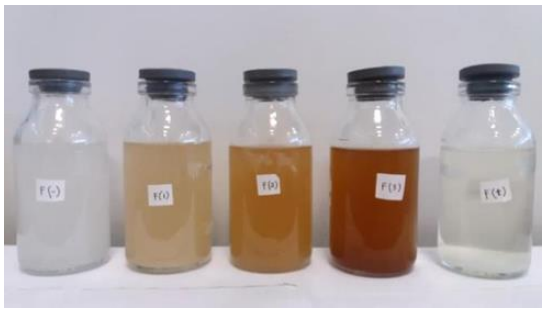

Figure 3 The results of making emulsion preparations of telang flower extract (Clitoriaternatea)

In this research, the emulsion preparation of Bunga Telang (Clitoriaternatea) extract was made in 5 different formulations, called F0, F1, F2, F3 and F4. Each emulsion made contains 10%, 15%, and 20% active ingredients of telang flower extract which are also used as the water phase with tween 80. The Virgin Coconut Oil (VCO) is used as oil phase and emollient, the basic nature of Virgin Coconut Oil (VCO) which is formed from high lauric acid (Lestari et al, 2013) for providing mosturizier and antioxidant properties then the preparation is not easily damaged and rancid due to storage(Rahmawanty, Annisa and Sari, 2020). In formulated emulsion preparations, no coarse granules were found, then it can be concluded that sunscreen emulsion preparations of telang flower extract were homogeneous.

2.3 The Evaluation of Pomegranate Flower Extract Microemulsion

2.3.1 The Emulsion Type Test

The emulsion of telang flower extract (Clitoriaternatea) is an oil-in-water (w/o) emulsion type. The macroscopic test as shown in the figure 4 Emulsion type test on prepared glass Emulsion type test with microscope(10x10).

In the figure 4 the addition of methylene blue into the preparation shows that methylene blue is evenly dispersed into the preparation. Oil-in-water emulsion type is a formulation that is widely used in pharmaceutical and cosmetic industries. Oil-in-water emulsion is also the most appropriate formulation for general use as a utensil for active ingredients that are easily washed off with water.

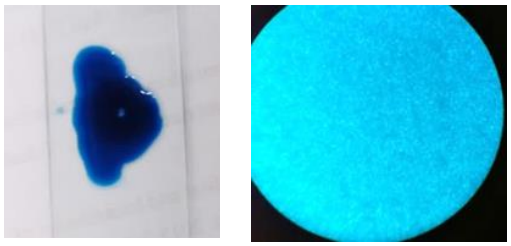

Figure 4. Emulsion Test

2.3.2 Stability Test

The stability test is conducted by freeze-thaw testing which aims to determine the stability of the preparation

during storage from time to time and ensure that the preparation will not change during storage within 1 year.

2.4 Organoleptic Test of Emulsion Preparation After Stability (Freeze Thaw)

Freeze thaw test aims to determine the stability during storage from time to time. The preparations were stored at cold temperatures and transferred to room temperature (20-25°C) and at low temperatures (4-20°C) for 6 cycles and observed once every 1 day.

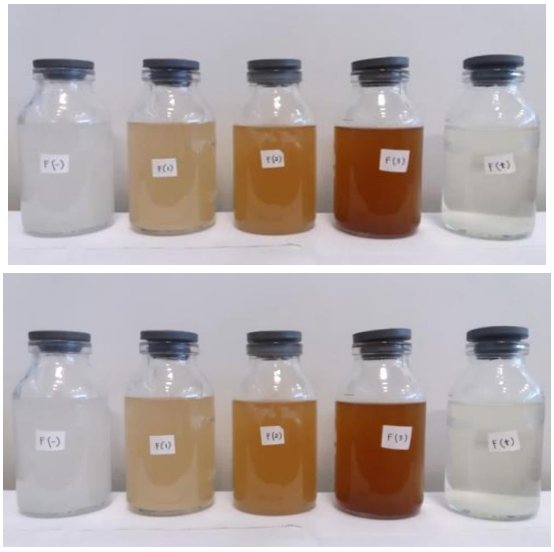

Figure 5. The organoleptic appearance of emulsion preparation of telang flower extract (Clitoriaternatea) after stability (freeze thaw).

Based on the figure above, the emulsion preparation of telang flower extract (Clitoriaternatea) was physically stable during storage ainces it did not change color, odor, clarity,and did not creaming and phaseseparation.

2.4.1 Viscosity Test

The measurements were made through a Brookfield Cone and Plate Viscometer. First, turn on the tool and prepared the appropriate cone. Then, the tested microemulsion preparation is put into a container (plate). The plate is positioned below the cone, the cone will be driven by a motor according to the selected speed. The microemulsion sample will be squeezed between the cone and plate. After the set was prepared, press the start button and wait for a few minutes until the viscosity value of the preparation appears.

The viscosity test of telang flower extract emulsion (Clitoriaternatea) was conducted using an Oswaltd viscometer which aims to determine the viscosity value of each sunscreen emulsion formulation and ensure that the expected specifications have been met. The viscosity test results can be seen in the following table:

Table 1. The results of viscosity test of telang flower extract emulsion (Clitoriaternatea)

Formulation	Viscosity (cPs)
F0	0.423 ± 0.021
F1	0.457 ± 0.007
F2	0.476 ± 0.012
F3	0.566 ± 0.013
F4	0.589 ± 0.004

Based on the table, it can be seen that the average viscosity value of emulsion sunscreen oftelang flower extract from three replications in five formulations. The formulation 0 (negative control) has a viscosity value of 0.423 cPs. The formulation 1 with a concentration of 10% telang flower extract has a viscosity value of 0.457 cPs. The formulation 2 with a concentration of 15% telang flower extract had a viscosity value of 0.476 cPs. The formulation 3

with a concentration of 20% Mengrove leaf extract had a viscosity value of 0.566 cPs. Lastly, the formulation 4 (positive control) has a viscosity value of 0.589 cPs.

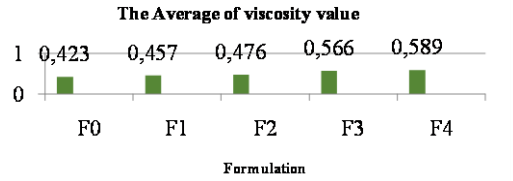

Figure 5. The graph of average value of emulsion preparation of telang flower extract viscosity (Rhizophora mucronate Poiret)

Diagram Description:
F0 = Negative control extract 0%
F3 = Telang flower extract 20%
F1 = Telang flower extract 10%
F4 = Positive control SPF 30
F2 = Telang flower extract 15%

Based on the figure above, it can be seen that there is a relationship between the concentration of telang flower extract (Clitoriaternatea) and the viscosity value of the preparation. From the results of data analysis, it is known that Kolmogorof-Smirnov test shows that giving different concentrations of telang flower extract in five formulations has a significant effect because it has a p.value of 0.728 > 0.05, which means it fails to reject H0 which means the data has a normal data distribution.Then, it continued with the homogeneity test using Levene test which got a p value of 0.207 > 0.05 which means the data is homogeneous.

It continued with one-way ANOVA test which obtained a p value of 0.000 <0.05, which means that there is a significant difference in giving the different concentrations of telang flower in five formulations. Furthermore, a post hoc Tukey HSD test was conducted to find out which formula had a difference.

Table 2. The results of double comparison test of viscosity value of solar preparation of telang flower extract (Clitoriaternatea) using Tukey HSD post hoc test

Formulation	Hypothesis	T test statistics	Sig (P.value: 5%)	Desc.
0	1	-.022333	.808	Same
	2	-.045667	.245	Same
	3	-.119667	.001	Difference
	4	-.156667	.000	Difference
1	2	-.023333	.784	Same
	3	-.097333	.005	Difference
	4	-.134333	.000	Difference
2	3	-.074000	.031	Difference
	4	-.111000	.002	Difference
3	4	-.037000	.422	Same
4	-	-	-	-

Description: The p.value is compared with 5% alpha significance level in Tukey HSD post hoc test

Based on the table above, it concluded that between multiple comparison tests using Tukey HSD post hoc, it shows that there is no difference in formulation 0 with hypotheses 1 and 2 because p.value is higher than 5% significance value, namely (0.808, and 0.245), whilethere is a difference between

formulation 0 and hypotheses 3 and 4 because the p-value is lower than significance value of 5%, namely (0.001 and 0.000).

Compared with hyphotesis 2, there is no difference in formulation 1 because p.value is higher than the 5% significance value, namely (0.784), whereas when compared with hypotheses 3 and 4 there is a difference because the p value is lower than the 5% significance value (0.005 and 0.000). In formulation 2 when compared with hypotheses 3 and 4, there are differences because p.value is lower than 5% significance value (0.031 and 0.002). Then, formulation 3 which is compared with hypothesis 4 has no difference because the p.value is higher than the 5% significance value (0.422).

Multiple comparison test using post hoc Tukey HSD concluded that there are 4 formulations which are the same, it means that there is no difference after giving telang flower extract, such as in formulation 0 with hypotheses 1 and 2, formulation 1 with hypothesis 2, and formulation 3 with hypothesis 4. Also, there are 6 formulations that are not the same, it means that there is a difference after giving the telang flower extract to viscosity value (in formulation 0 with hypotheses 3 and 4, formulation 1 with hypotheses 3 and 4, and formulation 2 with hypotheses 3 and 4).

2.5 Telang Flower Extract Sunscreen Potency Test Value % of ErythemaTransmission (%TE)

From the observational data of transmittance values at various wavelengths tested using a UV-Vis spectrophotometer, the percentage values of erythema transmission were obtained as follows:

Table 3. The result value % of erythema transmission (%TE)

Replication	The Result Value % of Transmission Erythema (%TE)				
	F0	**F1**	**F2**	**F3**	**F4**
1	2.678%	4.622%	4.544%	2.143%	3.093%
2	2.672%	4.620%	4.548%	2.151%	3.089%
3	2.669%	4.628%	4.548%	2.152%	3.101%
Mean ± Standard Deviation	2.68% ± 0.014	4.62% ± 0.004	4.55% ± 0.002	2.15% ± 0.005	3.09% ± 0.006
Category	Extra Protection	Extra Protection	Extra Protection	Extra Protection	Extra Protection

Based on the table 3, it can be seen that the average erythema transmission value (% TE) of 3 replications in five formulations was included in extra protection category, which means that the emulsion preparation of telang flower extract is able to protect normal and sensitive skin because it absorbs less than 95% of UV rays to prevent erythema and pigmentation. This is in accordance with the opinion of Kreps, Cumpelik and Goldenberg (1972) which states that a preparation is said to have extra protection

value because it has a UV transmission range of 1-6%.

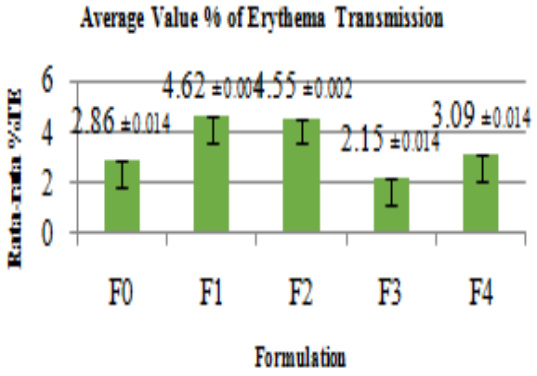

Figure 6 The graph of average %TE of emulsion preparations of telang flower extract (Clitoriaternatea).

Based on the picture, it can be seen that there is a relationship between the difference in the concentration of telang flower extract made in a concentration of 200 ppm in preparation and the average value of %TE, where the higher the concentration is, the smaller percentage of erythema transmission. This is in accordance with the opinionofMeisha(2018),which states that the higher the concentration of a given extract, the higher the ability to absorb light, then the light transmission to skin is less.

From the results of data analysis, it is concluded that Kolmogorof-Smirnov test shows that giving a different concentration of telang flower extract in five formulations has a significant effect of 0.255> 0.05, which means that it fails to reject H0 since the data has a normal data distribution. The homogeneity test using Levene test that has a value of 0.555 > 0.05, which means the data is homogeneous. The one-way ANOVA test which has a p. value < 0.05, which means that there is a significant difference in giving the different concentrations of telang flower in five formulations. If it is significant, then a multiple comparison test is conducted through Tukey HSD post hoc test to find out which formulas have differences and which do not have differences.

Table 4. The results of multiple comparison test value of erythema effect of telang flower extract (Clitoriaternatea) emulsion preparation using Tukey HSD post hoc test

Formulation	Hy po-thesis	T test statistics	Sig (P.value: 0.05)	Desc.
0	1	-1.950333*	0.000	Difference
	2	-1.873667*	0.000	Difference
	3	.524333*	0.000	Difference
	4	-.421333*	0.000	Difference
1	2	.076667*	0.000	Difference
	3	2.474667*	0.000	Difference
	4	1.529000*	0.000	Difference
2	3	2.398000*	0.000	Difference
	4	1.452333*	0.000	Difference
3	4	-.945667*	0.000	Difference
4	-	-	-	-

Description: The p.value is compared with the 5% alpha significance level in Tukey HSD test

Based on table 4, it concluded that there are differences between multiple comparison tests using post hoc Tukey HSD in all formulations because the p.value is lower than the 5% of significance value, which is 0.000. It can be concluded that all formulations of emulsion preparations of telang flower extract (Clitoriaternatea) have unequal values, it means that there is a difference after administration of telang flower extract on the effectiveness of erythema.

2.6 Value % of Pigmentation Transmission (%TP)

From the observational data of transmittance values at various wavelengths tested using a UV spectrophotometer, the percentage values of pigmentation transmission were obtained as follows:

Table 5 The results of % pigmentation transmission (%TP) emulsion preparations of telang flower extract (Clitoriaternatea)

Replication	Result Value % of Pigmentation Transmission (%TP)				
	F0	**F1**	**F2**	**F3**	**F4**
1	20.78%	29.63%	28.30%	15.34%	20.21%
2	20.77%	29.58%	28.27%	18.02%	20.21%
3	20.77%	29.60%	28.28%	17.99%	20.24%
Mean ± standard deviation	20.78% ± 0.007	29.60% ± 0.025	28.28% ± 0.152	17.12% ± 1.539	20.21% ± 0.000
Category	Total blocks (*Sunblock*)	Total blocks (*Sunblock*)	Total blocks (*Sunblock*)	Total blocks (*Sunblock*)	Total blocks (*Sunblock*)

Based on table above, it concluded that the average of pigmentation transmission value (%TP) of three replications in five formulations of emulsion preparations of telang flower extract (Clitoriaternatea) is included in Total Block (Sunblock) category, which means that the preparation of telang flower extract is able to protect normal, healthy and sensitive skin in maximum level to prevent the skin from erythema and pigmentation.

From the results of data analysis, it is known that Kolmogorof-Smirnov test showed that giving the different concentrations of telang flower extract in five formulations had a significant effect of 0.180 > 0.05, which means that it failed to reject H0 since the data has a normal data distribution.

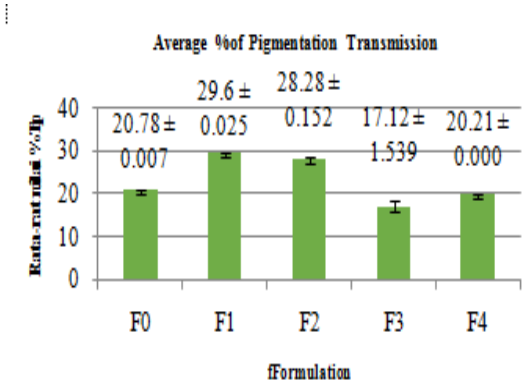

Figure 7 The graph of average %TP for emulsion preparations of telang flower extract (Clitoriaternatea)

The homogeneity test using Levene test that has a value of 0.00 < 0.05, which means that the data distribution is not homogeneous. The one-way ANOVA test which has a p value < 0.05%, which means that there are significant differences in the variation of the concentration of telang flower in five formulations. When it is significant, then a multiple comparison test is conducted using Tukey HSD post hoc test to find out which formulas have differences and do not have differences.

Table 6 The results of double comparison test of pigmentation effect value (%TP) using the Tukey HSD post hoc test

Formulation	Hyphotesis	T test statistics	Sig (P.value: 0.05)	Description
0	1	8.83000	0.000	Difference
	2	7.51000	0.000	Difference
	3	3.65667	0.001	Difference
	4	0.55333	0.856	Same
1	2	1.32000	0.207	Same
	3	12.48667	0.000	Difference
	4	9.38333	0.000	Difference
2	3	11.16667	0.000	Difference
	4	8.06333	0.000	Difference
3	4	3.10333	0.002	Difference
4	-	-	-	-

Description: The p.value is compared with the 5% alpha significance level in Tukey HSD post hoc test

Based on the table of multiple comparison test results in formulation 0 with hypotheses 1, 2 and 3 are having p.value<0.05, it can be interpreted that there are differences in each formulation. However, between the formulation 1 and hypothesis 4 has a p value > 0.05, which means there is no difference.

DISCUSSION

The results of phytochemical screening of flavonoid compounds in methanol extract of telang flower (Clitoriaternatea) showed positive resultsindicated by orange color. This identification of these flavonoids is through Willstater reagent where magnesium (Mg) and concentrated hydrochloric acid (HCl) react to form bubbles which are H2 gas. Besides, the concentrated Mg and HCl powder serves to reduce the benzopyron core contained in the flavonoid structure that indicates with an orange or red color (Senja, Issusilaningtyas and Setyowati, 2014).

The results of phytochemical screening in tannin test with FeCl reagent were indicated by a positive reaction when there was a green-black or blue-black color change. The methanol extract of the leaves (Clitoriaternatea) showed positive results indicated by a change in color sample to blackish green which is a non-polar condensed tannin(Utri, Dan and Islami, 2013).

The more the concentration of the telang flower extract added, it causes the increase of emulsion viscosity, an increase in viscosity value is possible because of the water content in its extract, the more of concentration extract, the bigger the water content that increases the viscosity of the emulsion(Sukma, 2018).

Then, it can be concluded that there is a relationship between in the concentration of telang flower extract (Clitoriaternatea) which made in a concentration of 200 ppm in emulsion preparations with an average value of %TP, where the higher the concentration is, the lower the percentage ofpigmentation transmission. This is in accordance with the opinion Alunpah (2018) which states that the higher the concentration of a given extract, the higher the ability to absorb light, then the transmitted light to the skin is minimum.

CONCLUSION

It can be concluded that telang flower extract can be used as a sunscreen to protect the skin from UV light since it has enoughemulsion preparation that minimize the light absorbation to the skin. Also, it has methanol extract that very beneficial for skin health.

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