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Study on Chemical Components and Antioxidant Activity of Cream Products Made of Telang Flower (*Clitoria ternatea* L.) and Honey

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ABSTRACT

Facial skin is most often exposed to UV rays and various disorders that need special creams with active ingredients containing antioxidant properties. This study aims to determine the chemical compounds of butterfly pea or telang flower (Clitoria ternatea L.*) and determine the antioxidant activity of its cream products. Six treatments consisted of ratio flower extract to honey were performed. The chemical components of telang flower were determined by GCMS method and antioxidant activity test using DPPH method. Results showed that major compound with retention at 23.989; 27.039; and 27.641 min were hexadecanoic acid, oleic acid, and octadecanoic acid with area percentage of 32.70%; 28.92%; and 15.42%, respectively. In addition, there were 5 minor compounds including palmitoyl chloride with retention 28.710 min and area of 7.07%, glycidyl palmitate (retention 18.767 min, area of 5.76%), Heptacosanol (retention 22.818 min, area of 3.01%), Nitroisobutylglycerol (retention 18.059 min, area of 4.50%), and Hydroxymethylfurfural (retention 14.324 min, area of 2.61%). Based on the antioxidant activity test, the highest antioxidant activity results were found in cream formulation 1 (F1) with an* IC_{50} *of 3.906 µg/mL, followed by cream formulation 2 (F2) which has antioxidant activity after of 6.532 µg/mL.*

1. INTRODUCTION

Butterfly pea flower (*Clitoria ternatea* L.), locally known as "telang" flower, is one of the special herbal plants because all parts from roots to flowers are believed to have medicinal properties (Marpaung, 2020). Telang flower have many benefits, one of which acts as an antioxidant because it has high anthocyanins (Hariadi *et al*., 2018; Fangoboi *et al*., 2023; Kusumanti *et al*., 2023; Purwanto *et al*., 2022). Anthocyanins exhibit not only antioxidant properties, but also antimicrobial, anti-inflammatory, antioxidant, anti-allergic, anti-viral, and many other health benefits (Khoo *et al*., 2017; Kharade, 2023). Recently, it was also reported that telang flower can be used as an alternative for antihypertention (Rizkawati *et al*., 2023), antimicrobial (Widyasanti & Febrianti, 2024), and natural die for soda drink (Unawahi *et al*., 2022).

The antioxidant and antibacterial properties of telang flowers can be utilized as a natural ingredient in skincare products such as facial skin. Skin is an organ that covers the outer body and functions as a protector from various kinds of disturbances and stimuli. The skin is sensitive, with a complex and elastic epithelial structure. Facial skin is the most visible and also the most easily exposed to the sun and dust. UV rays from sunlight have the most harmful impact on the skin because the reactions they cause can adversely affect the skin, such premature aging and decreased immune response ability (Sari, 2015). Excessive exposure to UV radiation can form reactive oxygen species or ROS that can damage cell membranes, proteins, carbohydrates and nucleic acids that trigger oxidation (Mansur *et al.,* 2016; Haerani *et al.,* 2018). The mechanism of antioxidant compounds when inhibiting the production of ROS is by directly

cleaving and reducing the oxidants in and around cells, then avert ROS from reaching biological targets, restrict the spread of oxidants such as those that occur during lipid peroxidation and thwarting oxidative stress. This process prevents premature aging of the skin (Haerani *et al.,* 2018). Efforts to care for and maintain facial skin to avoid free radicals is by using skincare that contains antioxidants that can prevent exposure to free radicals (Riha *et al.*, 2021).

The skincare can be made using natural ingredients that contain antioxidants such as Telang flower and honey. Besides Telang flower, honey is also a natural ingredient rich in antioxidants and has antimicrobial performance. Honey contains glucose oxidase, glucose peroxidase enzymes, catalase, non-enzymatic contents such as amino acids, organic acids, carotenoids, proteins, Maillard reaction products, and more than 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives that support the antioxidant properties of honey (Sumarlin *et al*., 2014). The honey used in this study is trigona honey, from the bee species *Trigona* sp. Trigona honey has a higher antioxidant content than honey from the genus *Apis*. The chemical content in trigona honey is complex such as glucose, fructosam minerals and vitamins. Trigona honey contains trehalulose disaccharide which is an isomer of sucrose and is good for health because it has a low glycemic index and insulinemic index (Fletcher *et al*., 2020).

This research aims to find out the content of chemical compounds from telang flowers and to find out the antioxidant activity of facial creams product with a combination of telang flower extract and honey and to obtain the best formulation of the cream based on antioxidant activity. This research is important to determine the effect of the combination of telang flower and honey as a natural ingredient for antioxidant-rich cream products.

2. RESEARCH MATERIALS AND METHODS

Telang flowers were obtained from local garden in Kedaton, Bandar Lampung City (Figure 1). Trigona honey was obtained from Suhita Bee Farm. Additional raw materials were propylene glycol, stearic acid, cera alba, vaselin alba, triethanolamine, methyl paraben, and distilled water as ingredients for making face cream. The next materials are DPPH (2,2 DIiphenyl 1-1 Pickrylhydrzyl) solution and pure vitamin C as antioxidant activity analysis materials. Telang flowers were studied for their chemical content such as antioxidant compounds and other constituent compounds using GCMS (QP2010S SHIMADZU). Antioxidant activity test was conducted on the facial cream that has been made using a combination of telang flower extract and honey. Antioxidant activity test using DPPH method. The tool used in antioxidant activity test is UV-VIS spectrophotometer.

Figure 1. Butterfly-pea or telang flower (left), and its extract (right)

2.1. GCMS Analysis

GCMS analysis of telang flower was conducted at Vahana Scientific Laboratory, Padang City. The analysis refers to the Vahana Scientific Laboratory method in 2023. Samples of fresh telang flowers were weighed 1.00 grams and then dissolved with 2-propanol 5 ml. The dissolved sample was filtrate with a 0.22 µm PTFE syringe filter. The next step is injected into the GCMS system as much as 0.5 µL, then evaporated until the sample cup turns into vapor or gas. The gaseous sample is carried by a carrier gas with a constant flow rate into the separator.

2.2. Product of Facial Care Cream Combination of Telang Flower Extract and Honey

Telang flowers that have been tested for chemical components using GCMS are utilized as ingredients of facial creams. Telang flowers were extracted using the maceration method and the extract was used for facial cream ingredients, combined with honey. The dosage of 100% cream is 10 g. The formulation of facial care cream combined with telang flower extract and honey is presented in Table 1.

The use of stearic acid in cream making is commonly used because stearic acid is a cream base along with Triethanolamine (TEA). The use of stearic acid had a concentration requirement of 1 - 20%. Stearic acid functions as an emulgator and can be used to neutralize the cream when combined with TEA. The use of TEA in cream making is as an emulsifier, mixer or binder of oil and water. The concentration of TEA used in creams for emulsification is 2 - 4%. The use of propylene glycol in cream functions as a moisture lock and can function as a preservative. Propylene glycol is commonly used in cream products so it was used in this study. The use of cera alba in the cream as a good oil and night so that it can produce a homogeneous dosage mass. Cera alba can also be used as an emulgator, increasing viscosity and improving consistency and stabilizing cream products. Vaseline alba is used in the product of creams as an emollient and base, as well as a moisturizer. Methyl paraben is used in cream products as a preservative and functions as an antimicrobial. The concentration of methyl paraben is 0.02 - 0.03%. Distilled water is used as a solvent in the product of cream products.

Table 1. Face cream dosage formulation of combination of telang flower extract and honey

Materials	Treatment (Ratio of flower extract to honey)					
	F0 (0%:0%)	**F1 (20%:0%)**	**F2 (15%:5%)**	**F3 (10%:10%)**	**F4 (5%:15%)**	**F5 (0%:20%)**
Telang flower extract (g)	**0**	**2.0**	**1.5**	**1.0**	**0.5**	**0**
Trigona honey (g)	**0**	**0**	**0.5**	**1.0**	**1.5**	**2.0**
Propylene glycol (g)	0.8	0.8	0.8	0.8	0.8	0.8
Stearate acid (g)	1.5	1.5	1.5	1.5	1.5	1.5
Cera alba (g)	0.2	0.2	0.2	0.2	0.2	0.2
Vaseline alba (g)	0.8	0.8	0.8	0.8	0.8	0.8
Triethanolamin (g)	0.15	0.15	0.15	0.15	0.15	0.15
Methyl paraben (g)	0.083	0.083	0.083	0.083	0.083	0.083
Aquadest (ml)	4.467	4.467	4.467	4.467	4.467	4.467

Note: Face cream product of different combinations of telang flower extract and honey at a ratio of flower extract : honey of F0 (0%: 0%); F1 (20%: 0%); F2 (15%: 5%); F3 (10%: 10%); F4 (5%: 15%); and F5 (0%: 20%).

2.3. Antioxidant Activity Test using DPPH Method

The antioxidant activity was tested using DPPH method (Kusumanti *et al*., 2023) conducted at the Integrated Research and Testing Laboratory of Gadjah Mada University, Yogyakarta, referring to the test method of the Integrated Research and Testing Laboratory of Gadjah Mada University.

3. RESULTS AND DISCUSSION

3.1. GCMS Analysis of Telang Flower

Figure 2 depicts extract collected from telang flower. The chromatogram test results of telang flower are presented in Figure 3. The composition of telang flower based on GCMS test is presented in Table 2. In Table 2, there are 8 peaks with major compound levels at retention 23.989; 27.039; and 27.641 min namely for hexadecanoic acid, oleic acid, and octadecanoic acid with a percent area of respectively 32.70%; 28.92%; and 15.42%. The characterization results of the major compounds of telang flower are presented in Figure 3 (a), (b), and (c). The hexa-decanoic acid ($C_{16}H_{32}O_2$) is the IUPAC name of palmitic acid, which is a saturated fatty acid that is also often called hexadecyclic acid and cetylic acid. Palmitic acid has strong antimicrobial and antioxidant properties. In addition, palmitic acid also functions as an important component in cell membranes and lipid transportation processes. The second major compound, oleic acid, is an unsaturated fatty acid that belongs to the omega-9 fatty acid group.

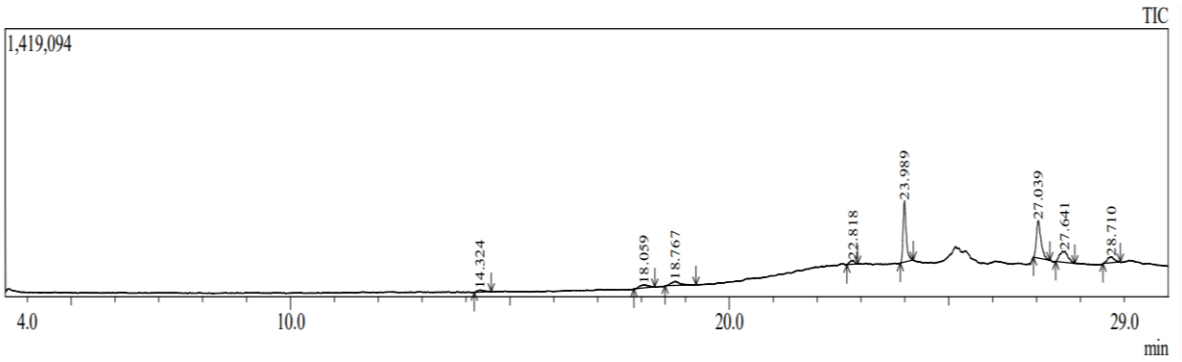

Figure 1. Chromatogram of-butterfly pea or telang flower

Table 2. Composition of telang flower based on GCMS test

Peak	Retention Time	Initial Time	Final Time	Area	Area %	Height	Height %	Name
1	14.324	14.190	14.575	129615	2.61	11847	1.73	Hydroxymethylfurfural
2	18.059	17.825	18.300	223118	4.50	16570	2.41	1.3-Propanediol, 2-(hydroxymethyl)-2-nitro
3	18.767	18.535	19.240	285479	5.76	23240	3.39	Glycidyl palmitate
4	22.818	22.680	22.920	149393	3.01	20233	2.95	1-Heptacosanol
5	23.989	23.895	24.190	1621115	32.70	324106	47.22	n-Hexadecanoic acid
6	27.039	26.930	27.300	1433572	28.92	198155	28.87	Oleic Acid
7	27.641	27.440	27.870	764167	15.42	59131	8.61	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
8	28.710	28.515	28.910	350564	7.07	33118	4.82	Palmitoyl chloride

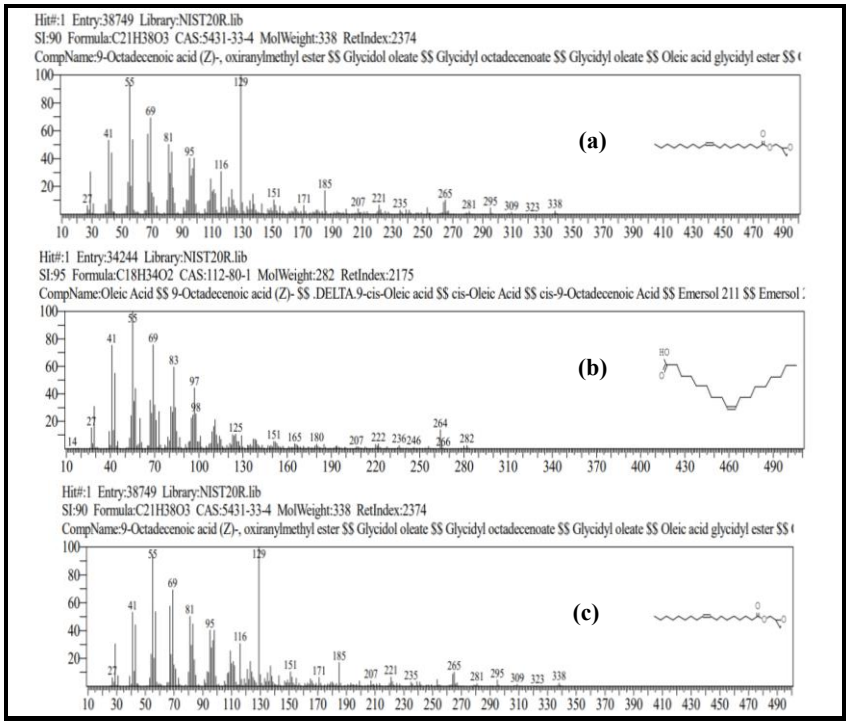

Figure 3. Characterization results of telang flowers on height of the compound: (a) 47.22%; (b) 28.87%; (c) 8.61%

Some studies show that oleic acid has anti-inflammatory effects that can help reduce inflammation in the body. Octadecanoic acid or better known as stearic-acid is a type of long-chain saturated fatty acid be composed of 18 carbon atoms (C). The major components of telang flower can be seen in Table 2.

In addition to the 3 major compounds, there are 5 minor compounds that make up the telang flower. The results of the characterization of minor compounds of telang flower are presented in Figure 4 (a), (b), (c), (d), and (e). The first compound, palmitoyl chloride, is a transparent to yellowish liquid that is used in many applications, especially in the cosmetic industry. Palmitoyl chloride is a fatty acid derivative that is widely used in the synthesis of lipids and various other compounds. Then the compound glycidyl palmitate has the chemical formula $C_{19}H_{36}O_3$ with a molecular weight of 312.5 g/mol. Glycidyl Palmitate is a chemical used for the manufacture of lysophosphatidic acid which inhibits apoptosis. Furthermore, the compound heptacosanol or heptacosan-1-ol has the chemical formula $C_{27}H_{56}O$ with a molecular weight of 396.7 g/mol. Heptacosanol is a primary fatty alcohol that has a very long chain, namely heptacosane, where one of the terminal methyl hydrogens has been replaced by a hydroxy group. Then the Nitroisobutylglycerol compound is a compound that is proven to inhibit the growth of microorganisms. The last minor compound, Hydroxymethylfurfural or HMF, is basically a fraction of sucrose and fructose. The palmitoyl chloride was captured at a retention of 28.710 min with a percent area of 7.07%. The next minor compound is glycidyl palmitate which is caught at a retention of 18.767 min with a percent area of 5.76%. Furthermore, the Heptacosanol compound

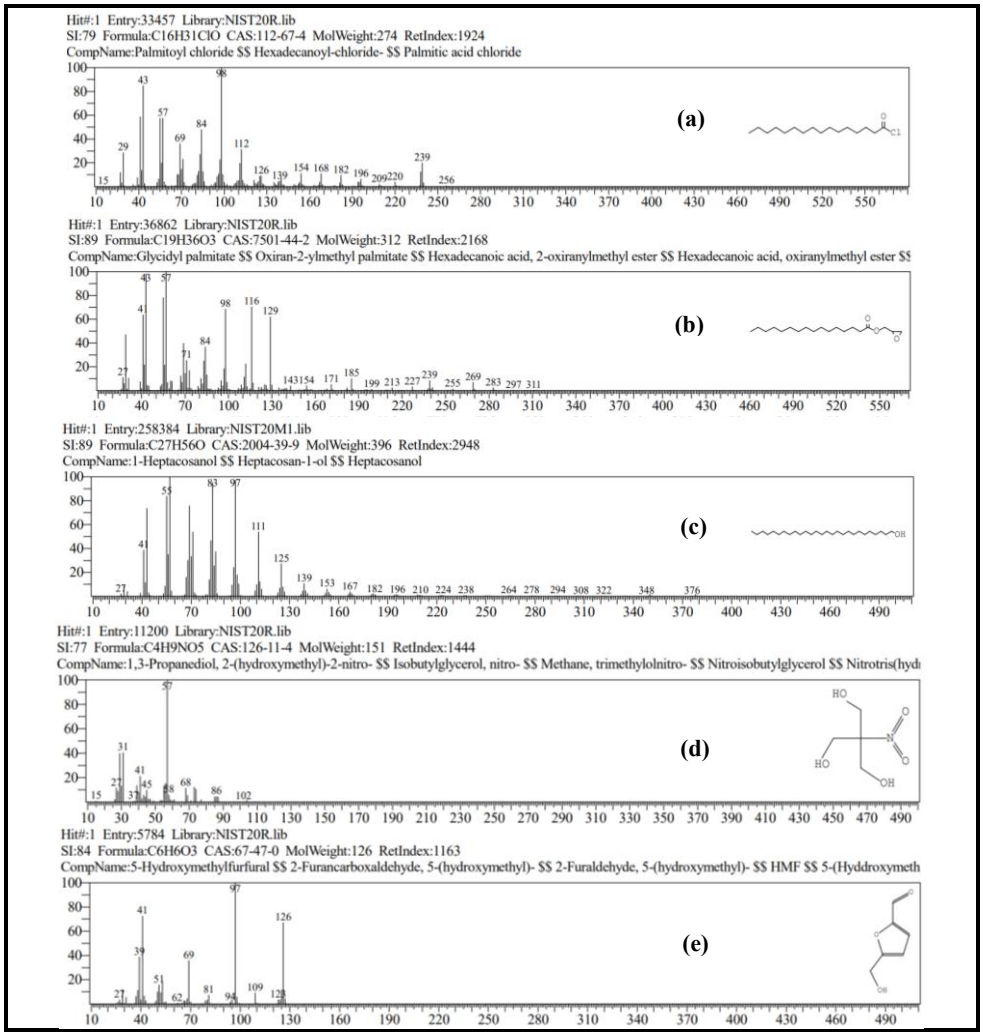

Figure 4. The results of the characterization of minor compounds of telang flowers in height of the compounds: (a) 4.82%; (b) 3.39%; (c) 2.95%; (d) 2.41%; and (e) 1.73% (e).

was captured at a retention of 22.818 min with a percent area of 3.01%. The next minor compound is Nitroisobutylglycerol which is captured at a retention of 18.059 min with a percent area of 4.50%. The last minor compound is Hydroxymethylfurfural which is captured at a retention of 14.324 min with a percent area of 2.61%. The area shows the size of the telang flower component compound.

3.2. Combination Cream Product of Telang Flower and Honey

The product of face cream with a combination of telang flower extract and honey uses formulated ingredients such as telang flower extract, honey, propylene glycol, stearic acid, cera alba, vaseline alba, triethanolamine, methyl paraben and distilled water. The cream was prepared by mixing the oil phase and water phase. The first stage was done by melting the oil phase (cera alba, stearic acid, and vaseline alba) and the water phase (methyl paraben, propylene glycol, triethanolamine and aquadest) at 70ºC. After the ingredients melted completely, the water phase was mixed into the oil phase then stirred using a mortar and stemper for 4 minutes until a cream base was formed. The cream base that has been formed is allowed to stand until the temperature becomes 40ºC. After the temperature of the cream base stabilized, telang flower extract and honey were added and stirred until homogeneous. The difference in the color of the facial care cream produced based on different concentrations of telang flower extract addition are visible in Figure 5.

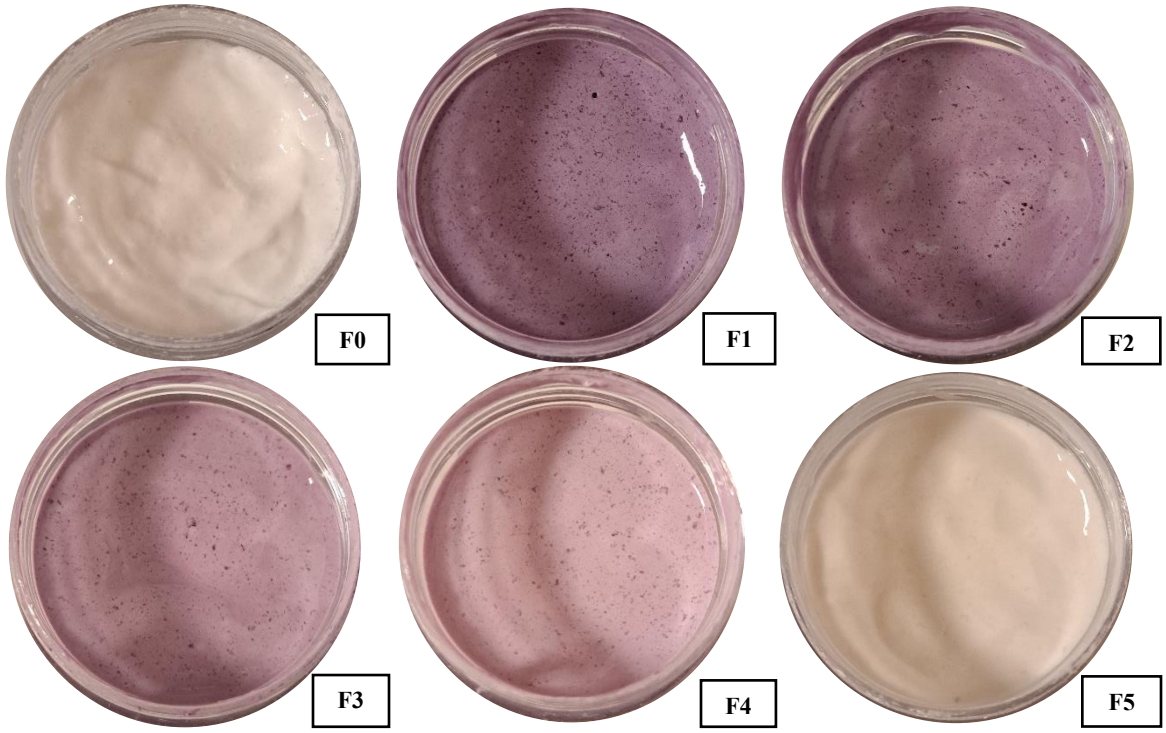

Figure 5. The facial cream product with different combination of telang flower extract and honey.

The most striking characteristic of telang flowers is their blue color, which is due to the anthocyanins they contain. Anthocyanins are one of the components of flavonoids. There are 3 mechanisms of flavonoids work; hinder energy metabolism, hamper cell membrane function, and impede nucleic acid synthesis (Thaliarinanta *et al*, 2024). Inhibition by flavonoids is the inhibition of the formation of free radical compounds in the skin so that damage to the skin does not occur. Anthocyanins in telang flowers are anthocyanins that have more than two acyl groups or are called polyacylated. Anthocyanins that are polyacylated are more stable when compared to anthocyanins that do not have acyl groups (Marpaung, 2020). Polyacylated anthocyanins in telang flowers are more stable in capturing free radicals caused by UV radiation so that the skin is better preserved.

In the formulation of facial care cream, adding in telang flower extract affects the color of the cream product. The higher the concentration of telang flower extract addition will increase the density of the cream color to purplish blue, as well as the addition of honey to the cream. This happens because telang flowers have a deep blue color due to the high anthocyanin content (Thaliarinanta *et al*, 2024). The addition of honey makes the cream product yellowish white, so the more honey is added to the cream product, the more yellowish white the cream color will be.

3.3. Antioxidant-Activity Test Using DPPH Method

Antioxidants are compounds that under low concentration conditions can inhibit or prevent the oxidation of substrates in chain reactions. Antioxidants can donate their electrons to free radical molecules, so antioxidants can stabilize free radicals and stop the chain reaction (Kurniadi *et al.*, 2024). Free radical molecules caused by oxidative stress play a role in the skin aging process. Excessive oxidative stress due to an imbalance of the antioxidant defense system and uncontrolled production of free radicals derived from oxygen can cause damage including to the skin (Haerani *et al.*, 2018). Antioxidant activity test on the face care cream combination of telang flower extract and honey was conducted using DPPH method with vitamin C control. The results of the antioxidant activity test are presented in Table 3. The DPPH method is performed to neutralize 50% of radical compounds. This method is commonly known as the calculation of inhibitory concentration or IC_{50}. The smaller the IC_{50}, the more effective an active substance is in counteracting free radicals (Marpaung, 2020). More specific, a compound is said to have a powerful antioxidant if the IC_{50} value is less than 50 ppm, strong for IC_{50} values of 50-100 ppm, and weak if the IC_{50} value is 151-200 ppm.

Table 3. The result of antioxidant activity test

Treatment	Result	Unit
Control (Absorbic acid/Vitamin C)	3.757	µg/mL
F0 (0% telang flower extract and 0% honey)	4.849	%
F1 (20% telang flower extract and 0% honey)	3.906	µg/mL
F2 (15% telang flower extract and 5% honey)	6.532	µg/mL
F3 (10% telang flower extract and 10% honey)	9.521	µg/mL
F4 (5% telang flower extract and 15% honey)	11.458	µg/mL
F5 (0% telang flower extract and 20% honey)	26.628	%

The antioxidant activity is measured by determining the IC_{50} value using a linear regression formula using the line equation $y = ax + b$. The calculation is done by entering the y value ($y = 50$), and the x value is obtained as the IC_{50} value (Agusthi & Romadhan, 2024). Based on the antioxidant activity test, the highest free radical capture activity is obtained in F1 or the face care cream product with a combination of 20% telang flower extract and 0% honey which has an IC_{50} antioxidant activity at 3.906 µg/mL. In F2, a combination of 15% telang flower extract and 5% honey that has the second highest antioxidant activity after F1 at 6.532 µg/mL. In F3, face care cream product with combination of 10% telang flower extract and 10% honey has a free radical capture activity at 9.521 µg/mL. Followed by F4, which is a combination of 5% telang flower extract and 15% honey with IC_{50} at 11.458 µg/mL.

F0 and F5 facial cream products cannot be calculated IC_{50} because up to the maximum volume added with DPPH, F0 is only able to counteract 4.849% free radicals, and F5 is only able to counteract 26.628% free radicals. Both did not cut off at a point above 50%. In most studies, the antioxidant activity of telang flower was found to be lower than Vitamin C in counteracting free radicals (Marpaung, 2020). According to Rajamanickam *et al.* (2015), in their research stated that the methanol extract of telang flower has an IC_{50} level of 95.30 µg/mL. When compared to Vitamin C, the IC_{50} is only 70.80 µg/mL which states that Vitamin C is more effective in counteracting free radicals. According to Jannah *et al.* (2022), it was also mentioned that the dried telang flower sample showed an IC_{50} of 126.80 ppm (µg/mL) and when compared to Vitamin C which has an IC_{50} of 1.85 ppm (µg/mL), Vitamin C is much more effective in counteracting free radicals.

This statement is in accordance with the results of the study, that Vitamin C is stronger in antioxidant properties than telang flower extract in cream products, but not too far from the level of effectiveness. In this study, F1 face care cream product showed IC_{50} antioxidant activity of 3.906 µg/mL. When compared to Vitamin C which shows an IC_{50}

of 3.757 µg/mL, the effectiveness of Vitamin C and F1 is not much different, but Vitamin C is still more effective in warding off free radicals. Face care cream products with a combination of telang flower extract and honey F1, F2, F3, and F4 show antioxidant properties that are still classified as strong, because the level of antioxidant strength with the DPPH method is <50 µg/mL for the very strong category. As for F0 and F5, we can only see the percentage of antioxidant activity because the effectiveness is not up to 50%. The antioxidant properties of telang flower are influenced by its antioxidant bioactive components which are included in the flavonoid group. In the study, the addition of telang flower extract greatly influenced the antioxidant activity of the cream preparation. This shows the strong antioxidant properties of telang flower. The addition of honey to the cream preparation did not have much effect on antioxidant activity, but it can still be antioxidant even though it is weak. So it is known that the antioxidant properties of honey are not as strong as telang flower.

4. CONCLUSION

Based on the research-that-has been done, it is known that-the chemical compounds of telang flower based on GCMS analysis are 3 major compounds at retention of 23.989; 27.039; and 27.641 minutes in the form of hexadecanoic acid, oleic acid, and octadecanoic acid with a percent area of 32.70%; 28.92%; and 15.42%. Based on antioxidant activity test, the best treatment was found in F1 cream product with IC_{50} value of 3.906 µg/mL.

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