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FORMULATION AND ANTIOXIDANT ACTIVITY TEST OF PEEL *OFF* GEL MASK KOMBUCHA BUNGA TELANG USING THE DPPH METHOD

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ABSTRACT

Free radicals are unpaired molecules that can cause damage to body cells. This cellular damage can be countered with natural antioxidants derived from plants, one of which is butterfly pea flower, when combined with kombucha, resulting in butterfly pea kombucha that has better antioxidant activity for the body. Peel-off gel masks are facial cosmetic preparations that have a gel-like consistency when applied to the face. After a certain period, they dry and form a film layer that can be peeled off. The concentrations of butterfly pea flower kombucha used were 5%, 7.5%, and 10%. The purpose of the study was to determine the antioxidant activity of the butterfly pea flower kombucha peel-off gel mask using the DPPH method and to obtain the optimum formula. The DPPH method was used to assess the ability of the antioxidant activity to inhibit DPPH. The evaluation of the peel-off gel mask formulation was conducted through organoleptic testing, homogeneity, pH, spreadability, adhesion, drying time, viscosity, and flow properties. To determine antioxidant activity, antioxidant activity testing was performed using a UV-Vis spectrophotometer. The results of the evaluation of the peel-off gel mask formulations 1, 2, and 3 fulfill all testing requirements, with antioxidant activity showing IC_{50} values of 56.03 ppm ± 0,02 for Formula 1, 52.50 ppm ± 0,02 for Formula 2, and 49.32 ppm ± 0,04 for Formula 3
Keywords: Kombucha, Butterfly Pea Flower, Peel-Off Gel Mask, Antioxidant, DPPH.

INTRODUCTION

Cell damage caused by free radicals can be addressed with the presence of antioxidants from within or outside the body. Antioxidants play role to neutralize free radicals in the body, and they are divided into two categories: natural and synthetic. Natural antioxidants are safer for the body and come from vegetables, fruits, and plants found in the surrounding environment. (Safnowandi, 2022).

One of the natural antioxidants is the butterfly pea flower. The butterfly pea flower, commonly known as blue pea flower, is a climbing plant that is often found around homes, near rice fields, or in plantations. Butterfly pea flower belongs to the Fabaceae family. (Marpaung, 2020).

The utilization of butterfly pea flowers as a main ingredient in the cosmetics world is very important to develop. The ethanol extract of butterfly pea flowers contains metabolite compounds such as: flavonoids,

tannins, alkaloids, saponins, and anthocyanins. The research by Andriani & Murtisiwi (2020) indicates that the 70% ethanol extract of butterfly pea flowers has very strong antioxidant activity with an IC_{50} value of 41.36..

In addition, natural antioxidants come from fermentation, specifically kombucha. Kombucha is one of the conventional biotechnology products that is being widely researched at the moment. Kombucha is a drink obtained from the fermentation of tea by microbes consisting of bacteria and yeast. This microbial mixture can form a strong symbiosis that is capable of inhibiting pathogenic bacteria. The fermentation process of kombucha leads to the formation of cellulose polymer pellicles due to the activity of specific strains originating from bacteria such as *Acetobacter sp*. (Villarreal-Soto *et al*., 2018). Kombucha does not only come from the fermentation of black tea, but butterfly pea flower tea can also be made into kombucha with very strong antioxidant activity, with an IC50 value of 11.143 ppm after a fermentation period of 6 days at a temperature of 30°C (Wahyuningtias *et al.*, 2023).

A mask is a topical cosmetic preparation used on the face that comes in the form of powder, paste, or liquid, and is then left to dry or react with ingredients that can improve skin conditions by producing a skin-tightening effect as a cleanser. One type of mask is the peel-off gel mask, which has a gel-like consistency. Once it dries, it can be peeled off due to a film layer made from polyvinyl alcohol (PVA) (Nofita *et al.*, 2023)

This research aims to formulate a peel-off gel mask of butterfly pea flower kombucha at concentrations of 5%, 7.5%, and 10%, and to conduct antioxidant activity testing using the DPPH method.

METHODS

Tools and Materials

The tools used in this research are Homogenizer (IKA), analytical balance (OHAUS), pH-meter (Metler Toledo), viscometer *Brookfield RV* (AMETEK), spectrophotometry UV-Vis (Shimadzu UV mini-1240) and other laboratory equipment.

The materials used in this research are scoby and bacterial starter culture (bought from Rumah Fermentasi), Simplisia butterfly pea flower (bought from Rempah Burisma), aquadest (CV mustika Lab), polyvinyl alcohol (PVA) (CV Pratama Sains Global), glycerin, methyl paraben, propyl paraben, Carbopol 940, TEA (CV mustika Lab), DPPH (PT himedia), methanol proanalysis (CV mustika Lab), vitamin C (PT Merck), hydrochloric acid, magnesium powder,

Dragendorff's reagent, and Liebermann-Burchard reagent (CV mustika Lab).

Research Path

1. Plant determination

Plant taxonomy was determined at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, FMIPA Universitas Padjajaran, with number No.38/HB/04/2024.

2. Manufacturing process of butterfly pea kombucha

Simplicia of dried butterfly pea flowers Dried butterfly pea flowers (*Clitoria ternatea L*) of 20 grams are steeped in a solution of 1000 ml of water while stirring over medium heat for 15 minutes, then filtered to separate the extract from the butterfly pea flowers. Add sugar with a concentration of 10% (w/v) or 100 grams, filter again, and then cool to a temperature of 25°C within no more than 4 hours. Next, the cooled butterfly pea tea is transferred into a transparent glass jar, and 10% starter or 100 ml is added to the jar along with the SCOBY. Then tie it with a rubber band covered with cloth and avoid sunlight; the fermentation process is carried out for 6 days at a temperature of 30°C. (Wahyuningtias *et al.*, 2023).

3. Phytochemical screening

Phytochemical screening is used to assess the presence of secondary metabolite compounds in butterfly pea flower kombucha, including flavonoids, tannins, saponins, alkaloids, and steroids/terpenoids. (Abdilah et al., 2022).

2. Formulation of Peel-off Gel Mask

Table 1. Formula of butterfly pea flower kombucha Peel-Off Gel Mask

Material	**Function**	**Formula Masks Gel *Peel Peel-Off* (%)**			
		F0	**F1**	**F2**	**F3**
Butterfly pea kombucha	Active substance	-	5	7,5	10
Polyvinyl alcohol	Film Formation	13	13	13	13
Carbopol 940	Gelling agent	0,4	0,4	0,4	0,4
Glyserin	Humectan	5	5	5	5
TEA	Alkalizing agent	0,4	0,4	0,4	0,4
Propylparaben	Preservative	0,02	0,02	0,02	0,02
Methyilparaben	Preservative	0,2	0,2	0,2	0,2
Oleum rosae	Fragrance	Qs	Qs	Qs	Qs
Aquadest	Solvent	ad 100	ad 100	ad 100	ad 100

The preparation of a kombucha butterfly pea flower peel-off gel mask is carried out by first developing PVA and Carbopol 940. Next, PVA and Carbopol 940 are mixed in a homogenizer. Then add glycerin, methylparaben, propylparaben, and TEA into the homogenizer. After that, slowly add butterfly pea flower kombucha at concentrations of 5%, 7.5%, and 10%. The remaining aquadest is placed into the

homogenizer, stir until homogeneous. *(Khoirunnisa et al.*, 2022).

3. Evaluation of Peel-Off Gel Mask

3.1 Organoleptic test

The evaluatio was performed using sensory analysis, focusing on the color, shape, and scent of the peel off gel mask formulation (Rizikiyan et al., 2023).

3.2 Homogenity test

The testing was conducted by weighing 500mg of the peel-off gel mask preparation, which was then applied to a glass slide. A solution is said to be homogeneous if no clumps of particles are found and the texture is uniform (Setiawati & Sukmawati, 2019).

3.3 pH measurement

A total of 500mg of peel-off gel mask preparation is dissolved in 10ml of aquadest. Then, the electrode part of the pH meter is immersed. The pH value is displayed on the screen (Rizikiyan *et al*., 2023).

3.4 Spreading ability test

A total of 1 gram of peel-off gel mask preparation is placed between two glass plates. Add a weight of 150 grams on top of the glass, let it sit for 1 minute, then measure the diameter using a caliper (Adhayanti *et al*., 2022).

3.5 Inherent ability test

A total of 500 mg of the sample is placed on a glass slide and covered with another glass slide. Then a load of 250 grams is added for 1 minute, then the load is lifted and an 80-gram weight is added. Release the lever and record the time of release of the preparation from the glass slide. (Nofriyaldi *et al*., 2022).

3.6 Drying time test

A total of 500 grams of peel-off gel mask is applied to an area of 5x5 cm on the arm, and the time required for the preparation to dry so that it can be peeled off is measured (Setiawati & Sukmawati, 2019).

3.7 Viscosity measurement

The testing was conducted using a Brookfield RV viscometer. A total of 250 grams of the peel-off gel mask preparation was placed into a beaker glass, and the spindle was positioned inside until it was submerged. Then turn on the viscometer and observe the results. If the scale (%T) is below 10, then the speed (Rpm) must be increased; if the speed (Rpm) reaches 100, then the spindle should be replaced with a larger number (Zubaydah *et al*., 2020).

3.8 Flow properties test

The flow property test is conducted starting from the lowest speed (rpm) and then moving to the highest speed (rpm). The flow properties can be determined by creating a curve between the speed (rpm) and the force (dyne/cm^2), resulting in a graph of force (x)

versus speed. (y) (Zamzam & Indawati, 2018).

4. Antioxidant activity test

4.1 Manufacture and measurement of DPPH solution

A total of 10mg of DPPH powder is then mixed with 100ml of methanol with a concentration of 100ppm (Surharyani *et al*., 2022).

Then the DPPH Master Solution with a ratio of 3ml DPPH concentration of 100 ppm and 4ml methanol will be measured for maximum wavelength absorption of 400-800 nm wavelength and carried out *Operating Time* over a time period of 0-30 minutes with time intervals of every 2 minutes (Dewi Zulfa *et al*., 2023)

4.2 Measurement of antioxidant activity of telang flower kombucha

The stock solution of kombucha telang flowers the stock of kombucha butterfly pea flowers was prepared by diluting 10 mL a liquid with ethanol a final volume of 100 mL, rsulting in a concentration pf 100 ppm. Then a dilution solution of 5, 10, 15, and 20 ppm is made which is taken from the parent solution. Next, 3ml is taken and 4ml of methanol is added to each distillation. After that, the tube containing the sample is incubated for 10 minutes in a dark place (Wahyuningtias *et al.*, 2023).

4.3 Antioxidant activity reduction Gel *Peel Off* Mask kombucha bunga telang

Stock solution The solution of the peel off gel mask containing kombucha bunga telang with a concentration of 5%, 7.5% and 10%. Were prepared by dissolving 10 mg of each formulation in 100 mL of methanol to obtain a final concentration of 100 ppm. Then a dilution solution of 2, 3, 4, and 5 ppm is made which is taken from stock solution. Next, 3 mL is taken and mixed with 4 mL of methanol. After that, the tube containing the sample is incubated for 10 minutes in a dark place. Then the absorbance of *the peel off gel mask of* kombucha telang flowers with concentrations of 5%, 7%, and 10% was measured using a UV-Vis spectrophotometer.

Data Analysis

Formula for determining antioxidant activity:

$$\%\ Inhibisi = \frac{abs\ blanko - abs\ sampel}{abs\ blanko}\ x\ 100\%$$

IC50 is the concentration of a test substance that can inhibit free radical activity (DPPH) up to 50%. To get an IC score$_{\text{of 50}}$

It can be determined by calculating based on the percentage of inhibition of each concentration by continuing to find the linear regression value of the formula as follows:

$$y = bx + a$$

Information:
y= Percent free radical capture (50)
b= Slope
x= Sample concentration
a= Y axis curve cut-off point *(intercep)* (Surharyani et al., 2022)

Regression analysis is a mathematical model that can be used to determine the relationship between two or more variations. Regression analysis aims to predict the value of a variable (dependent/bound variable) through other variables (independent/independent variables). The greater the value of r2, the better the bound variable. The magnitude of the r value *Square* between 0 and 1(Rizikiyan & Pandanwangi, 2019).

RESULTS AND DISCUSSION

1. Making kombucha bunga telang

The result of making kombucha bunag telang is 20 grams in 1000ml of water with the addition of 10% or 100 grams of sugar and the addition of 10% starter and soby. The telang flower kombucha produced 900ml out of 1000ml is due to the filtration process a lot of water is left behind so that it is not optimal.

2. Phytochemical screening

The results obtained from the table above are secondary metabolite compounds produced from flavonoids, alkaloids, saponins and terpenoids. These results are in line with the research conducted *(Abdilah et al*., 2022) metabolite compounds produced by alkaloids, flavonoids, saponins and tannins.

Table 2. Phytochemical screening results

Testing	Result	Information
Flavonoids	+	Red Solution
Alkaloids	+	There is a precipitate
Tannins	-	Chocolate solution
Saponins	+	Foamed
Terpenoids	+	Formed violet

3. Formulation evaluation

Figure 1. Results of telang flower kombucha peel off gel mask

The results of making peel *off gel masks with* telang flower kombucha with different concentrations of 5%, 7%, and 10%.

Table 3. Organoleptis results

Sample	Texture	Color	Smell
F0	Soft	White	*Oleum rosae*
F1	Soft	Light blue	*Oleum rosae*
F2	Soft	Dark blue	*Oleum rosae*
F3	Soft	Purplish blue	*Oleum rosae*

Based on the results of organoleptis testing gel masks *peel off* Kombucha Bunga Telang that the preparation has a different concentration for each formula, then the organoleptis results of the color experience differences for a concentration of 5% light blue, a concentration of 7.5% dark blue and a concentration of 10% purplish blue, for the texture and smell of almost all of them.

Table 4. Homogeneity test results

Sample	Homogeneity
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Results from homogeneity testing of gel mask preparations *peel off* Kombucha Bunga Telang has no insoluble ingredients, all materials are soluble and homogeneous.

Table 5. pH measurement results

Sample	Average ± SD
F0	5.88±0.302
F1	5.66±0.062
F2	5.51±0.02
F3	5.24±0.01

Results from pH testing of gel mask preparations *peel off* Kombucha bunga telang is for the highest pH, namely formula 1 concentration of 5% with an average value of pH 5.66, and the lowest pH value of formula 3 concentration of 10% with an average value of pH 5.24 with the difference in pH measurement results caused by the concentration of kombucha bunga telang as an active ingredient. Based on the pH results of each formula meets the skin pH requirements, which is 4.5-6.5 (Rizikiyan *et al., 2023)*

Table 6. Spreading ability

Sample	Average (cm) ± SD
F0	5.98±0.076
F1	5.54±0.066
F2	5.80±0.079
F3	5.94±0.07

Based on the results obtained from the gel mask preparation *peel off* Kombucha bunga telang meets the requirement of 5-7cm (Rizikiyan *et al., 2023)*. The spread testing of each formula has increased due to the difference in kombucha in each formula, the consistency of the formula is thicker in the preparation, the smaller the spread. The decrease in dispersion occurs through the size of the molcule unit because there has been solvent absorption so that there is an increase in resistance to flow (viscosity) which affects the ability to spread (Rompis *et al.*, 2019).

Table 7. Adhesion results

Sample	Average (seconds) ± SD
F0	00.08.53±0.043
F1	00.09.32±0.070
F2	09.09.01±0.128
F3	00.07.48±0.045

Results of each gel mask formula *peel off* Kombucha Bunga Telang meets the adhesion requirements of gel masks *peel off*

i.e. more than 4 seconds (Nofriyaldi *et al*., 2022). According to research Arman *et al.*, (2021) Between viscosity testing and adhesion testing there is a relationship, namely as the viscosity of semi-solid preparations increases, it will be followed by an increase in adhesion (Arman *et al.*, 2021)

Table 8. Drying time results

Sample	Average (minutes) ±SD
F0	19.34,83±0.136
F1	17.45.54±0.064
F2	18.31,20±0.076
F3	20.24,16±0,070

The results obtained based on the table above that gel masks *peel off* Kombucha Bunga Telang meets the requirements of the drying time of the gel mask *peel off* 15-30 minutes (Setiawati & Sukmawati, 2019). The formula that dries quickly is formula 1 because of the small concentration of active ingredients. The difference in drying time in each formula is affected by the active ingredient and the high concentration of PVA requires a long drying time.

Table 9. Viscosity results

Sample	Average (cPs) ± SD
F0	34780 ± 840
F1	46266.7±1404.75
F2	44667 ± 833
F3	28000 ± 250

The results of the viscosity test based on the table above that from each gel mask preparation formula *peel off* Kombucha bunga telang meets the viscosity requirement of around 2,000-50,000 cPs (Zubaydah *et al*., 2020). The lowest viscosity is in formula 3 and the highest viscosity is in formula 1. This viscosity value is inversely proportional to the value of spread power because the higher the value of spread, the lower the viscosity value and vice versa.

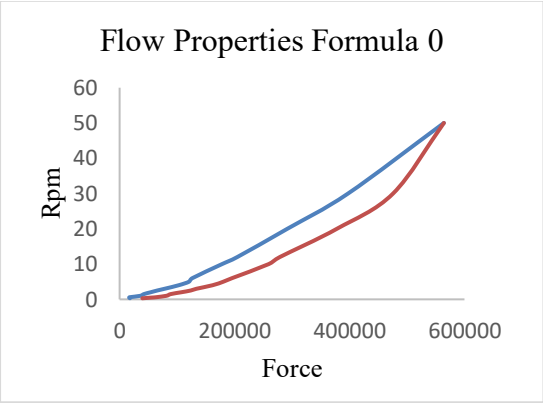

Figure 2. Base flow properties curve

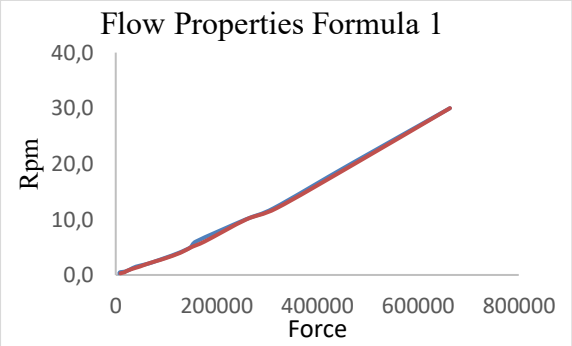

Figure 3. Formula 1 flow properties curve

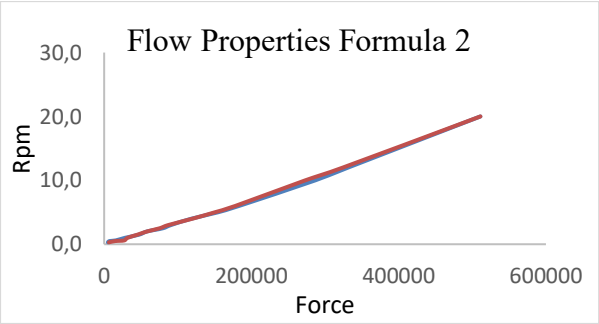

Figure 4. Formula 2 flow properties curve

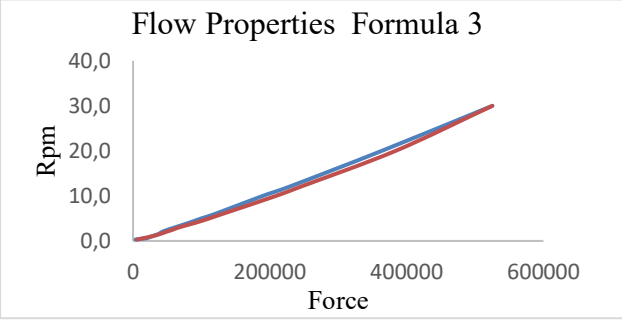

Figure 5. Formula 3 flow properties curve

The results obtained from the flow property test show that the base and formula 3 are classified as antiticsotropic flow types. Then for the results of formula 1 and formula 2 are classified into the thixotropic stream. Ticsotropic is a flow property that is quite consistent in the container, but it is easy to pour and to return to the original state takes a short time with the downward curve to the left of the ascending curve. Antiticsotropics are the opposite of ticotropics where the structure of the sole will become a gel when left unattended will return to the sole (Hardani et al., 2021). This is due to the influence of flow type on the dosage form, the influence of the PVA base material which is too large and the length of the test time that affects the flow properties.

4. Antioxidant activity

The determination of the maximum wavelength (λ) obtained was around 514.5nm absorbance and *a stable operating time* of 0.691 at 5 minutes with a time interval of 2 minutes with a stable absorbance value of 0.673.

Table 10. Results of IC50 antioxidant activity

Sample	Average (IC50) ±SD
Vitamin C	4.36±0.00
Kombucha Bunga Telang	14.18±0.00
F1	56.03±0.02
F2	52.58± 0.02
F3	49.32±0.04

The results of the DPPH activity test for the maximum wavelength obtained were 514.5 nm with an absorbance of 0.691 and *the operating time* of DPPH with DPPH stability reacted for about 10 minutes for the incubation process.

The results obtained from antioxidant activity, namely the IC50 value of vitamin C, which is 4.36, are classified as very strong antioxidants because vitamin C has been proven to have high antioxidants compared to the preparation of Kombucha Bungatelang peel off gel mask and has been used as a daily antioxidant. For the preparation of gel *mask peel off* kombucha telang flower formula 1,2 and 3 with

concentrations of 5%, 7.5% and 105 had different antioxidant activities with IC50 values of 56.03 ppm, 52.58 ppm, and 49.32 ppm respectively. For formulas 1 and 2, including strong antioxidants (IC50: 50-100) as well as formula 3, and kombucha telang flowers are classified as very strong antioxidants (IC50 < 50 ppm) (Caption *et al., 2021)*.

CONCLUSIONS

Based on the conclusions of this study,The results of the evaluation of the preparation of kombucha gel *peel off mask in* the flower meet the requirements for the evaluation of peel off gel masks. The preparation of peel off *gel mask* kombucha with kombucha has antioxidant activity with an IC50 value, a concentration of 5%, an IC50 value with an average of 56.03 ppm ± SD 0.02, a concentration of 7.5% of the IC50 value with an average of 52.5 ppm± SD 0.02, a concentration of 10% of the IC50 value with an average of 49.32 ppm ± SD 0.04, for Formula 1 and 2 it is classified as strong antioxidant activity while Formula 3 is very strong.

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