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FORMULATION AND ANTIOXIDANT ACTIVITY FACE TONER BUTTERFLY PEA FLOWER KOMBUCHA (*Clitoria ternatea* L.) WITH DPPH METHOD

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ABSTRACT

Antioxidants can be given to the skin through cosmetics such as face toners. One source of antioxidants is obtained through the fermentation of butterfly pea flower kombucha (*Clitoria ternatea* L.), which has been proven to have better antioxidant activity than butterfly pea flower extract alone. This study aimed to determine the metabolite compounds in butterfly pea flower kombucha and create a facial toner formulation with good antioxidant activity.
The butterfly pea flower kombucha was fermented for 6 days at room temperature. Facial toners were made with concentrations of 5%, 7,5% and 10%. Evaluation of the face toner includes organoleptic, homogeneity, pH, specific gravity, and viscosity tests. The antioxidant activity test used the DPPH (*1,1-diphenyl-2-pycrilhydrazil*) method, with vitamin C as a positive control. The results showed that there were alkaloids, flavonoids, saponins, and terpenoid compounds in butterfly pea flower kombucha. Face toners with concentrations of 5%, 7.5%, and 10% could be prepared and met all evaluations (organoleptic tests, homogeneity, pH, specific gravity, and viscosity). Antioxidant test results on formula 1 IC_{50} 50,21±SD 0,01 (strong), formula 2 IC_{50} 44,32±SD 0,02 (very strong) and formula 3 IC_{50} 38,62±SD 0,03 (very strong). The higher the concentration of butterfly pea flower kombucha, the higher the antioxidant activity. Butterfly pea flower kombucha (*Clitoria ternatea* L.) which is formulated as a face toner preparation has strong to very strong antioxidants, and the increasing concentration of active ingredients had an effect on antioxidant activity.

Keywords: face toner, butterfly pea flower kombucha, DPPH method, antioxidant activity

INTRODUCTION

Free radicals are found in many human lives, both from the environment and inside the body itself. One of the most easily observed effects of free radicals is premature aging; therefore, antioxidants are required. Generally, antioxidants can be obtained from natural ingredients that contain bioactive compounds such as flavonoids, alkaloids and terpenoids (Purwanto, Bahri and Ridhay, 2017). Butterfly pea flowers are natural ingredients that contain carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, and essential oils (Al-Snafi, 2016; Cahyaningsih, Era Sandhi, and Santoso, 2019), where flavonoids and anthocyanins are included in antioxidants. Kombucha is also a research area that is currently being developed, namely, a probiotic drink in the form of fermented tea using a symbiosis of bacteria and yeast known as SCOBY (Symbiotic Culture of Bacteria and Yeast) (Jayabalan *et al.*, 2014). SCOBY consists of the bacteria *Acetobacter xylinum* and fungi from the genus *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, and *Brettanomyce*. Kombucha tea has various health benefits, including immune booster, energy booster, improved human digestion,

antioxidant, antidiabetic, and anticancer effects (Susanti *et al.*, 2023). Kombucha is generally made from black tea/green tea, but can also be added to other natural ingredients that are high in phenolic compounds, such as ground cherry leaves (Rindiani and Suryani, 2023), soursop leaves, betel leaves, bay leaves, guava leaves, and coffee leaves (Wistiana and Zubaidah, 2015).

The advantage of the kombucha fermentation method is that it can extract phenolic compounds better than ethanol extract, as proven by research Wahyuningtias, Fitriana and Nawangsari, (2023), with the best kombucha fermentation time on the 6th day which has an IC_{50} value of 11.143 ppm, which is smaller when compared to the results of the antioxidant activity test in the study Andriani and Murtisiwi, (2020), regarding 70% ethanol extract of butterfly pea flowers which has an IC_{50} value of 41.36 ppm. The smaller the IC_{50} value, the higher the antioxidant activity of butterfly pea flower kombucha. The reference for determining the concentration was based on Listiyono (2023), regarding the antioxidant activity of facial wash gel with ethanol extract of butterfly pea flowers at concentrations of 5%, 7%, and 9%, where the highest antioxidant test results were obtained with a concentration of 9%, so that the concentration range of the test in this study was increased to 2.5%, where the concentrations tested were 5%, 7.5%, and 10%. The face-toner formulation was chosen because it is a liquid or water-like product with the same consistency as the butterfly pea flower kombucha (the active ingredient). If this face toner formulation succeeds and has antioxidant activity, then the butterfly pea flower kombucha would have wider possibilities to be formulated in other skincare/cosmetic formulations.

RESEARCH METHODS

This research is experimental research where butterfly pea flower kombucha was fermented for 6 days at room temperature and formulated in facial toners were made with concentrations of 5%, 7,5% and 10%. Evaluation of the face toner includes organoleptic, homogeneity, pH, and viscosity tests. The antioxidant activity test used the DPPH (1,1-*diphenyl*-2-*picrilhydrazil*) method, with vitamin C as a positive control. The resulting data from the face toner evaluation were analyzed using SPSS Ver 22.

Equipment and Materials

The equipment used in this research was an electronic kitchen scale SF-400, mercury and room thermometer, laboratory glassware, analytical balance (Corporation Model CP214), Ostwald viscometer, magnetic stirrer (Model SH-2), UV-Visible Spectrophotometer (Shimadzu UV Mini 1240), and a pH meter (Mettle Toledo).

The materials for this research is SCOBY (Symbiotic Culture of Bacteria and Yeast) and starter kombucha from Rumah Fermentasi (Jakarta), simplisia of butterfly pea flower kombucha from Toko Rempah Bu Risma (Bekasi), aquadest (CV. Bratacco), granulated sugar, technical Chloroform (PT Global Lab), technical Sulfuric Acid (PT Global Lab), technical Mayer reagent (PT Global Lab), technical Dragendorf reagent (PT Global Lab), technical Magnesium (PT Global Lab), technical concentrated HCl (PT Global Lab), technical $FeCl_3$ (PT Global Lab), technical anhydrous acetic acid (PT Global Lab), technical Propylene Glycol (PT Global Lab), technical Glycerin (PT Global Lab), DMDM Hydantoin (CV Pratama), technical TEA (PT Global Lab), tissue, aluminum foil, DPPH p.a (Himedia), Methanol p.a (CV. Mustika Lab) and butterfly pea flower kombucha.

Research Procedure

1. Collecting Butterfly Pea Flower

   Simplisia of butterfly pea flower is obtained from Toko Rempah Bu Risma (Bekasi) that has permit from Dinas Kesehatan (DINKES P-IRT 2103216080333-27) and Halal MUI (ID32110000576810922) with expire date before December 2024.
2. Plant Determination

Plant determination was performed at the Laboratory of Plant Morphology in the Biology Department of the University of Padjadjaran Bandung. The identification is to ensure that the incorrect plant used in this research is avoided.

3. Fermentation Tools Preparation
   A glass jar container for fermentation was prepared using the boiling method (Kamelia *et al.*, 2023).
4. Preparation of Butterfly Pea Flower Kombucha
   Brewing 20 grams of dried butterfly pea flowers in 1 liter of mineral water (aqua) at a temperature of 80-90 ^{o}C, for 15 minutes. The brewed water was filtered to separate the flowers from the tea water. Add 10% sugar (b/v) until dissolution, and put the tea water into a glass jar. Subsequently, the tea was allowed to cool to a temperature of 25 $^{\circ C}$. The cooling time did not exceed 4 hours. Then, 10% of starter kombucha and nata (SCOBY) with a size of ± 8 cm was added. Covering with cloth and tied with rubber. It was then fermented at room temperature for six days and was not exposed to direct sunlight (Modification from (Wistiana and Zubaidah, 2015; Khanifah, 2022).
5. Phytochemical Screening of Starter Kombucha adn Butterfly pea Flower Kombucha
   Phytochemical screening aims to determine the presence of secondary metabolites in the starter kombucha and butterfly pea flower kombucha.
   a. Flavonoid tests were carried out on 3 ml starter kombucha that was placed in a Test tube and 2-3 drops of 96% ethanol were added and evaporated until dry. Then 0.1 grams of Mg powder, and 10 ml of concentrated HCl were added (Nintiasari and Ramadhani, 2022). Flavonoid tests were carried out with different methods for 10 ml butterfly pea flower kombucha and then 5 ml aquadest was added into a Test tube and boiled for 5 minutes. The solution was filtered and concentrated HCl and Mg powders were added. If the solution shows Yellow, red, and orange indicate the presence of flavonoids (Wijaya, Paendong and Abidjulu, 2014).
   b. Alkaloid Test were carried out on 2 ml of starter kombucha and 2 ml of butterfly pea flower kombucha were put into a test tube, then 2 ml of ammonia and 2 ml of chloroform were added, then filtered and 10 drops of concentrated H_2SO_4 were added. The mixture was shaken and left to form 2 layers. The H_2SO_4 layer was transferred into 3 test tubes to be tested with Mayer, Dragendorff and Wagner reagents. The occurrence of white (for Mayer), orange-red (for Dragendorff), and brown (for Wagner) precipitates indicated the presence of alkaloids (Rauf Himaniarwati and Saranani, 2023).
   c. Saponin tests were carried out on 5 ml starter kombucha and 5 ml butterfly pea flower kombucha in each sample Test tube, and then 5 mL of hot water was added. Shake for 1 to 2 minutes and 2 drops of HCl 1 N were added until permanent foam was formed (the foam did not disappear for 7 minutes) (Wijaya, Paendong, and Abidjulu, 2014).
   d. The tannin Test was carried out on 2 ml of kombucha starter and 2 ml of butterfly pea flower kombucha in each test tube to be mixed with 2 drops of 1% $FeCl_3$ solution. A dark blue or greenish-black color indicates the presence of tannins (Wijaya, Paendong and Abidjulu, 2014).
   e. The steroid/terpenoid Test was carried out on 2 ml of starter kombucha and 2 ml of butterfly pea flower kombucha, which were evaporated in an evaporator cup. mL of chloroform, and anhydrous acetic acid (0.5 mL) were added. Concentrated H_2SO_4 was then added to the tube walls. A brownish-red or violet color at the solution border indicates the presence of triterpenoids, whereas a greenish-blue color indicates the presence of steroids (Abdilah *et al.*, 2022).
6. Formulation of Butterfly Pea Flower Kombucha Face Toner
   The butterfly pea flower kombucha was filtered to filter the nata (cellulose formed in kombucha) and prevent an uneven solution. All the ingredients needed (glycerin, propylene glycol, DMDM hydrantoin, TEA, butterfly pea flower kombucha, and distilled water) were weighed according to the formula. Then, glycerin, propylene glycol, and

DMDM hydantoin were placed in a beaker glass and mixed together, and butterfly pea flower kombucha was added and stirred with a magnetic stirrer rod until homogeneous. Add distilled water according to the calculation of each formulation and finally add the pH regulator (TEA) until the pH of the face toner preparation matches the pH of the facial skin 4.5-6.0 (Modification from (Permata, Pratama and Kotimah, 2023).

Table I. Formulation of Butterfly pea flower Kombucha Face Toner

Materials	Consentration (%b/b)				Function
	Basis	F1	F2	F3	
Butterfly pea flower Kombucha	-	5	7,5	10	Active Ingredients
Glycerin	2	2	2	2	Humectans
Propylene glycol	3	3	3	3	Humectans
DMDM Hydantoin	0,6	0,6	0,6	0,6	Preservatives
TEA	-	q.s	q.s	q.s	pH Regulators
Oleum Citri	q.s	q.s	q.s	q.s	Fargrances
Aquadest ad	100 ml	100 ml	100 ml	100 ml	Solvents

Notes:
q.s (quantum satis) means adding as much of this ingredient as needed to achieve the desired result.

7. Evaluation of Face Toner Formulation (Day-0)
   a. Organoleptic Test
      Organoleptic tests were performed by observing the physical properties of the butterfly pea flower kombucha face toner in terms of color, solution form, and aroma (Permata Pratama and Kotimah, 2023).
   b. Homogeneity Test
      Homogeneity tests were carried out by dropping each face toner formulation on a glass object and visually observing by the panelist whether there was sediment or not. The homogeneous category if the observed formulation appears even and does not produce sediment (Indriastuti *et al*., 2023).
   c. The pH Measurement Test
      The pH measurement began with calibration of the pH meter. The electrode is then inserted into the container that consists of face toner preparation; then, the scale will move and wait until the number does not change. The test was performed thrice for each formulation (Sari *et al*., 2021).
   d. Viscosity Test
      Before measuring the viscosity, the density of the face toner preparation was determined, starting with weighing an empty pycnometer, which was then inserted and reweighed to determine the density using the formula (Saputra, Wicaksono, and Irsan, 2017).

$$\rho = \frac{(W2 - W1)}{Vp}$$

Notes:
ρ : density (g/ml)
W1 : weight of empty pycnometer (g)
W2: Weight of pycnometer with sample (g)
Vp: Volume pycnometer

Viscosity was measured using an Ostwald viscometer. The tube was filled with a certain amount of sample (temperature was set at 20.0 °C ± 0.1 °C) as stated by the manufacturer. The meniscus of the liquid in the capillary tube was adjusted to the upper limit line using pressure or suction. Both the filling and capillary tubes were opened so that the liquid could flow freely into the container. The time required for

the liquid to flow from the upper limit to the lower limit in the capillary tube is recorded (Depkes RI, 1995).

The viscosity formula is as follows:

$$\frac{\eta 1}{\eta 2} = \frac{t1 \,.\, \rho 1}{t2 \,.\, \rho 2}$$

(Rasyadi, 2018)

Notes:
η1 = viscosity of sample liquid (centipoice (cP))
η2 = viscosity of the reference liquid (aqueous)(centipoice (cP));
ρ1 = density in sample liquid (gram/mL)
ρ2 = Density in reference liquid (aquadest) (gram/mL);
t1 = Time of flow of sample liquid (seconds); and
t2 = Time of flow of reference liquid (aquadest) (seconds)

8. Antioxidants Activity Test with DPPH Method
   a. Preparation of DPPH Solution
      DPPH powder was weighed as much as 10 mg and put into a 100 ml measuring flask then added with methanol p.a to the limit mark to obtain a concentration of 100 ppm (Wahyuningtias, Fitriana and Nawangsari, 2023).
   b. Preparation of Vitamin C Solution
      Ascorbic acid powder (vitamin C) was weighed as much as 10 mg and placed into a 100 ml volumetric flask, and methanol p. a. was added to the limit mark to obtain a concentration of 100 ppm. Dilution was required for the sample concentrations of 2, 3, 4, and 5 ppm. The vitamin C solution was taken 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml into each different volumetric flask, then methanol p.a. were added until the volume of 10 ml.
   c. Preparation of Butterfly pea flower Kombucha Solution
      Ten milliliters of kombucha were taken from 6 days of fermentation was taken 10 ml into a volumetric flask, and methanol p.a. was added until the volume reached 100 ml and shaken until homogenization. Later, they were diluted to 4 different concentrations of 5, 10, 15, and 20 ppm. The butterfly pea flower kombucha solution was taken 0,5 ml, 1 ml, 1,5 ml and 2 ml into each different volumetric flask, then methanol p.a. were added until the volume of 10 ml.
   d. Preparation of Butterfly pea flower Kombucha Face Toner Solution
      Based on formulas 1, 2, and 3, 10 ml of each face toner solution was added to different volumetric flasks, and methanol p.a. was added until the volume reached 100 ml and shaken until homogenization. Dilution was required for sample concentrations of 20, 30, 40, and 50 ppm. Then, from each face toner solution (that was already diluted in methanol p.a.), 2 ml, 3 ml, 4 ml and 5 ml were added to each volumetric flask, and methanol p. a. was added until the volume reached 10 ml.
   e. Determinantion of DPPH Maximum Wavelenght
      DPPH solution (3 ml and mixed with methanol (4 ml in a cuvette to measure the absorbance in the wavelength range of 400–800 nm using a UV-Vis Spectrophotometer (Wahyuningtias, Fitriana and Nawangsari, 2023).
   f. Operating Time DPPH
      The The DPPH solu (and mixed with methanol (4 m (cuvettea measure the absorbance using a Uv-usingSa pectrophotometer. Measurements were performed at the maximum wavelength during the period time 0-30 minutes, with measurement intervals every 2 minutes (Wahyuningtias, Fitriana, and Nawangsari, 2023).
   g. Determination of Antioxidant Activity of Butterfly pea flower Kombucha

The butterfly pea flower kombucha concentration samples of 5, 10, 15 and 20 ppm were taken 4 ml and put into a brown vial, followed by the addition of 3 ml of 100 ppm DPPH solution (Wahyuningtias, Fitriana and Nawangsari, 2023). After that, incubation was carried out for 10 minutes at 37 °C and the absorbance was measured at a wavelength of 516 nm (Rosyada, Agustina and Faizah, 2023).

h. Determinantion of Antioxidant Activity of Butterfly pea flower Kombucha Face Toner

Face toner concentration samples of 20, 30, 40, and 50 ppm were were were taken 4 ml from each concentration and placed in a brown vial, together with 3 ml of 100 ppm DPPH solution. The mixed solution was incubated for 10 minutes at 37°C in a dark place and the absorbance was measured.

Data Analysis

Absorbance data obtained from each sample concentration of butterfly pea flower kombucha can be used in the following equation to determine % inhibition.

$$\%\ Inhibition = \frac{A_0 - A_s}{A_0}\ x\ 100$$

Notes:
A_0 = Control absorbance (DPPH +Aquadest)
A_s = Sample and DPPH absorbance

The % inhibition data can be used in a linear regression equation to determine the IC_{50} (Inhibition concentration 50%) (Vidyatama Kusmin *et al*., 2023).

$$Y = a + bX$$

Notes:
Y = %inhibisi (IC_{50})
X = Concentration
A = Intercept
B = Slope

RESULTS AND DISCUSSION

Plant Determination

The identification results indicated that the plant used was *Clitoria tenatea* L. from *the Fabaceae* family with determination nomor No.36/HB/04/2024.

Phytochemical Screening

Table II. Result of Phytochemical Screening of Starter Kombucha and Butterfly pea flower Kombucha

Compounds	Starter Kombucha	Butterfly pea flower Kombucha	References
Alkaloids	-	+	Orange-red precipitate for Dragendorff's reagent, white precipitate for Mayer's reagent & brown precipitate for Wagner's reagent (Rauf, Himaniarwati and Saranani, 2023).
Flavonoids	+	+	Yellow, red or orange (Nintiasari and Ramadhani, 2022)
Saponin	+	+	Stable foam for 7 minutes (Wijaya, Paendong and Abidjulu, 2014)
Steroid/	+	+	Steroids: Blue greenish

Terpenoid	(Terpenoid)	(Terpenoid)	Terpenoids: Red, Brownish red or violet at solution border (Puspitasari and Wulandari, 2017)
Tannin	+	-	Dark blue/black greenish (Wijaya, Paendong and Abidjulu, 2014)

Note: + = indicating presence of compound
- = indicating the absence of compound

Based on these results, both the starter kombucha and butterfly pea flower kombucha contained the same secondary metabolite compounds, including flavonoids, saponins, and terpenoids. However, different secondary metabolite compounds were found in both the kombucha samples. Alkaloids were only found in butterfly pea flower kombucha and Tannin was only found in starter kombucha. This result proves that the addition of butterfly pea flower into kombucha fermentation provides alkaloid compounds, that linked to the previous research Abdilah *et al.*, (2022), regarding butterfly pea flower kombucha contains alkaloids, flavonoids, saponins and does not contain steroids and tannins. The tannin compounds that were found in starter kombucha were likely from phenolic compounds (from the main ingredients of starter kombucha, which is green tea) that reacted when $FeCl_3$ was added (Jawa L. *et al.*, 2020), which link to previous research Muhsinin, Pertiwi and Zaelani, (2023), regarding green tea kombucha which positively contains flavonoid compounds, saponins, tannins, and steroids/triterpenoids. Tannin compounds may not be detected in the butterfly pea flower kombucha, which can be caused by the following: 1) tannin is classified as a polar compound that is soluble in polar solvents such as water and ethanol (butterfly pea flower kombucha fermentation process involves water and formed ethanol) (Nofita and Dewangga, 2021); 2) tannin oxidizes into theaflavin and thearubigin compounds in the fermentation process, which causes fluctuations in tannin levels (Rachmawati *et al.*, 2023); therefore, a more specific phytochemical identification test is needed to detect the presence of the compounds, such as UV-Vis Spectrophotometer and Follin-Ciocalteu (Fitriana and Rahmawati, 2024).

Evaluation of Face Toner Formulation

Table III. Evaluation of Butterfly pea flower Kombucha Face Toner (Day-0)

Face Toner	Organoleptic		Homogeneity	pH ± SD	Density (g/ml) ± SD	Viscosity (cPs) ± SD
	Colour	Smell				
Base	Clear	Citrus	Homogeneous fluid	5,22 ± 0,049	1,0062 ± 0,0003	0,979 ± 0,0039
Formula 1	Blue	Citrus	Homogeneous fluid	5,64 ± 0,036	1,0134 ± 0,0005	1,025 ± 0,0039
Formula 2	Purplish-blue	Citrus	Homogeneous fluid	5,52 ± 0,015	1,017 ± 0,0005	1,05 ± 0,0053
Formula 3	Purple	Citrus	Homogeneous fluid	5,39 ± 0,015	1,0208 ± 0,0005	1,077 ± 0,0082

Figure 1. Face Toner Formulation (From left to right: Base, Formula 1, Formula 2 and Formula 3)

Organoleptic tests were aimed at checking the smell, color, and shape of the face toner preparation in terms of appearance through observation with the five senses and to observe whether the face toner preparation passed the organoleptic parameters. The addition of butterfly pea flower kombucha to face toner preparation affected its color appearance; the higher the butterfly pea flower kombucha concentration, the more purple the face toner would be. The smell of the face toner is derived from citrus essential oil, with a small amount of sour smell from kombucha. However, after some time, the smell of citrus essential oil disappears, so it is not effective for long term storage. All face toner formulas were homogenous fluids with no insoluble particles, cloudy, and air bubbles. A liquid form is suggested because if the toner has a thick form, it can leave a sticky feeling when used, and reduce comfort when using the facial toner (Noor Malahayati and Nastiti, 2023). pH measurement tests were aimed at finding the skin's suitable pH for toner formulas, to reduce the possibility of skin irritation due to the natural acidic pH in the active ingredient butterfly pea flower kombucha (final pH after fermentation is 4,5) (Kushargina *et al.*, 2023). The average pH of the face toner base was 5,22 ± SD 0,049, formula 1 was 5,64 ± SD 0,036, formula 2 was 5,52 ± SD 0,015 and formula 3 was 5,39 ± SD 0,015. The average pH of formulas 2 and 3 was slightly acidic compared to formula 1 because the butterfly pea flower kombucha concentration was higher. TEA as a pH regulator was added in various concentrations to accommodate different kombucha concentrations, so the face toner pH will adjust to skin pH, which is between 4,5-6,5 (Asjur *et al.*, 2023).

Before the viscosity procedure, density tests were carried out using a pycnometer. The density test is very important because it affects the viscosity (density value is directly proportional to viscosity), where the lower the density of a sample, the lower its viscosity (Permanadewi *et al.*, 2021). The viscosity procedure was carried out using a Viscometer Ostwald, based on the result experiment and calculation using an equation, the base of face toner has an average viscosity of 0,979 cp ± SD 0,0039, formula 1 1,025 cp ± SD 0,0039, formula 2 1,05 cp ± SD 0,0053 and formula 3 1,077 cp ± SD 0,0082. When observed sequentially, an increase in viscosity was observed. The addition of active ingredients (butterfly pea flower kombucha) has been shown to increase the viscosity of face toner preparations, but still meets the viscosity requirement for non-aerosol preparations, which is below 150 cPs (Indriastuti *et al.*, 2023). The higher the viscosity value of a formula, the higher the consistency level of the preparation (Yuniarsih *et al.*, 2020). Statistical analysis was conducted on the pH and viscosity data using IBM SPPS Ver.22 to test the normality and homogeneity of the data. The pH and viscosity data were normally and homogeneously distributed, with significance values (>0.050). The results of the one-way ANOVA parametric test showed a significance level of 0.000, which met the requirements (sig. <0.050), indicating that the pH and viscosity data between the face toner preparation formulas are significantly different. Data analysis was continued with the Post Hoc LSD test, the results showed a significance value under (sig. <0.050), which means that there is a significant difference between the addition of active ingredient concentration and the face toner pH and viscosity. For the pH data, there was another factor besides the kombucha concentration, which was the addition of TEA as a pH regulator. The relationship between the addition of kombucha and TEA is inversely proportional: the higher the concentration of the butterfly pea flower kombucha in the formula, the more acidic the pH of the face toner, and the lower the TEA added to the formula, the more acidic the pH of the face toner.

Antioxidant Activity Test
Determination of DPPH Maximum Wavelenght

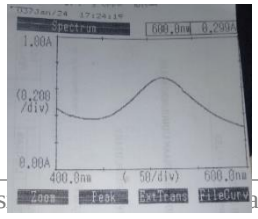

Figure 2. DPPH Maximum Wavelenght

The results of the maximum wavelength measurement (λmaks) with a UV-Vis spectrophotometer were 514.5 nm with an absorbance of 0.673.

Determination of Antioxidants Activity with DPPH Method

Table IV. Result of Antioxidant Activity Test

Sample	Concentration	Absorbance			%Inhibition			Average of IC_{50} (µg/ml) ± SD
		R1	R2	R3	R1	R2	R3	
Vitamin C	2 ppm	0,525	0,524	0,523	21,99	22,14	22,28	4,36 ± 0,0001
	3 ppm	0,460	0,460	0,460	31,65	31,65	31,65	
	4 ppm	0,342	0,342	0,342	49,18	49,18	49,18	
	5 ppm	0,296	0,296	0,296	56,02	56,02	56,02	
Butterfly pea flower kombucha	5 ppm	0,518	0,518	0,517	23,03	23,03	23,18	14,19 ± 0,0042
	10 ppm	0,439	0,439	0,439	34,77	34,77	34,77	
	15 ppm	0,305	0,305	0,305	54,68	54,68	54,68	
	20 ppm	0,222	0,222	0,222	67,01	67,01	67,01	
Base of Butterfly pea flower kombucha Face Toner	20 ppm	0,669	0,669	0,670	0,59	0,59	0,45	814,092 ± 38,132
	30 ppm	0,666	0,666	0,665	1,04	1,04	1,19	
	40 ppm	0,662	0,660	0,660	1,63	1,93	1,93	
	50 ppm	0,657	0,657	0,657	2,38	2,38	2,38	
Formula 1 Face Toner	20 ppm	0,655	0,654	0,655	2,67	2,82	2,67	50,21 ± 0,01
	30 ppm	0,565	0,565	0,565	16,05	16,05	16,05	
	40 ppm	0,431	0,431	0,431	35,96	35,96	35,96	
	50 ppm	0,344	0,344	0,344	48,89	48,89	48,89	
Formula 2 Face Toner	20 ppm	0,595	0,595	0,595	11,59	11,59	11,59	44,32 ± 0,02
	30 ppm	0,473	0,473	0,473	29,72	29,72	29,72	
	40 ppm	0,370	0,370	0,369	45,02	45,02	45,17	
	50 ppm	0,289	0,289	0,289	57,06	57,06	57,06	
Formula 3 Face Toner	20 ppm	0,544	0,544	0,544	19,17	19,17	19,17	38,62 ± 0,03
	30 ppm	0,413	0,413	0,412	38,63	38,63	38,7	
	40 ppm	0,326	0,326	0,326	51,56	51,56	51,56	
	50 ppm	0,218	0,218	0,217	67,61	67,61	67,61	

Furthermore, the base of the face toner preparation was also tested for antioxidants to ensure whether the ingredients used to make the preparation, affect on antioxidant activity and the test results obtained were that the base of the face toner had an average IC_{50} of 814.092 ppm ± SD 38.132 which was included in the very weak antioxidant category (more than 200 ppm) and the absorbance data obtained in **Table IV**, the base of the face toner has an absorbance that was not much different from the absorbance of the blank, so it can be said that it does not have much effect on the antioxidant value of the active ingredients (formula 1, 2 and 3). The antioxidant ability of the base likely comes from one of the excipients, namely propylene glycol, which has been proven through research Elisma, Lenggu and Takubessi, (2020) entitled 'Characteristics and Antioxidant Activity of Sweet Potato Syrup (Ipomea batatas (L) with Variations of Propylene Glycol' where the IC_{50} data (propylene glycol concentrations of 11%, 12% and 13% were respectively 142 ppm, 134 ppm, 128 ppm and 115 ppm) shows that the higher the concentration of propylene glycol, the greater the antioxidant power. The concentration of propylene glycol used in this study was only 3%, so the antioxidant effect of the face toner preparation was very weak and did not have a significant effect on the overall total IC_{50} value.

The average IC_{50} of formula 1 with 5% butterfly pea flower kombucha concentration is 50,21 ± SD 0,01, which is included in the category of strong antioxidants (50-100 ppm). Formula 2 with a concentration of 7.5% butterfly pea flower kombucha has an average IC_{50} of 44.32 ppm ± SD 0.02 which is included in the category of very strong antioxidants (<50 ppm). Formula 3 with a concentration of 10% butterfly pea flower kombucha has an average IC_{50} of 38.62 ppm ± SD 0.03 which is also included in the category of very strong antioxidants because its IC_{50} is less than 50 ppm. Compared to the average IC_{50} of vitamin C (positive control), which is 4.36 ppm ± SD 0.0001 (very strong antioxidant), face toner formulas are had weaker antioxidants, and since vitamin C were only used as validation samples to ensure that the research procedures used were correct. Compared to the average IC_{50} of butterfly pea flower kombucha which is 14.19 ppm ± SD 0.0042, the antioxidants in the face toner formulas were weaker due to the use of lower concentrations of butterfly pea flower kombucha in each formula (5%, 7.5% and 10%) compared to the concentration of butterfly pea flower kombucha tested (100%).

CONCLUSION

Butterfly pea flower kombucha has been proven to have secondary metabolite compounds including alkaloids, flavonoids, saponins, and terpenoids. Butterfly pea flower kombucha with concentrations of 5%, 7.5% and 10% can be formulated into face toner preparations and meets all preparation evaluation requirements (organoleptic, homogeneity, pH and viscosity of the preparation). All face toner formulas have antioxidant activity starting from formula 1 which has an IC_{50} value of 50.21 ppm ± SD 0.01 (strong), formula 2 of 44.32 ppm ± SD 0.02 (very strong), and formula 3 of 38.62 ppm ± SD 0.03 (very strong). The greater the concentration of butterfly pea flower kombucha added, the higher the antioxidant activity.

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