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FORMULATION AND ANTIOXIDANT ACTIVITY TEST OF FACE TONER EXTRACT PREPARATION FROM 70% ETHANOL OF CUCUMBER FRUIT (*Cucumis sativus* L.)

Meta Safitri, Sufyan Sauri, Banu Kuncoro, Arini Aprilliani

Department of Pharmacy, Universitas Muhammadiyah A R Fachruddin, Tangerang 15720, Indonesia

metaunimar@gmail.com

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ABSTRACT
Cucumber (*Cucumis sativus* L.) is a natural ingredient with potential therapeutic properties for addressing various skin-related problems. The study aims to identify a suitable formulation for a facial toner extract that exhibits favourable physical properties as determined by organoleptic, homogeneity, pH, and hedonic testing. The present study employed an experimental approach to produce a 70% ethanol extract of cucumber fruit through the maceration method, utilising 70% ethanol solvent. Subsequently, the extract derived from the cucumber fruit developed four formulas to prepare face toners. The concentrations of these formulas were designated as F0 (0%), FI (0.5%), F2 (1%), F3 (1.5%), and F4 (utilising brand x toner as a positive control). The physical evaluation test yielded the fulfilment of the physical criteria for the toner, encompassing the organoleptic, homogeneous, pH, and hedonic tests. The IC50 values obtained from the antioxidant activity test conducted on face toner preparations containing cucumber fruit extract at concentrations of 0.5%, 1%, and 1.5% were found to be 128 ppm, 91.017 ppm, and 62.218 ppm, respectively. The SPSS analysis reveals a significant difference in the IC50 value among the various formulas. Specifically, F3 exhibits a smaller IC50 value than the other formulas, indicating a stronger antioxidant activity. It is important to note that a smaller IC50 value indicates a stronger antioxidant activity. The study's findings suggest a positive correlation existing between the extract's concentration and its antioxidant activity, specifically, the extract denoted as F and administered at a concentration of 1.5%.

Keywords: Cucumber; Face toner; Antioxidant

1. INTRODUCTION

Individuals aspire to possess a hygienic, healthy, and meticulously maintained facial appearance. However, sometimes, the facial region encounters various issues, including a lacklustre and dishevelled appearance. A sense of insecurity may arise due to the lack of vibrancy in one's facial appearance. A facial freshener is necessary to cleanse and revitalise the countenance effectively to prevent a dull appearance. Toner is a liquid solution that removes residual dirt and makeup from the facial skin and provides a refreshing effect as well. Toner is a skincare product applied after the cleansing step and before applying facial moisturiser. Toners can eliminate impurities and purify facial skin while regulating sebum production without causing dehydration in delicate skin types. To mitigate facial oiliness, it is necessary to utilise a facial toner to impart a refreshed and immaculate appearance (Khanza & Mardhiyah, 2018)

Toner is a cleansing agent that is commonly retailed in cosmetic establishments, albeit at a relatively high cost. Because chemical toners can be costly, some women in their twenties and beyond who have oily skin may forego this particular facial treatment (Karyanto *et al.*, 2022). The toner available in the market is typically composed of alcohol and is marketed as a cosmetic product for removing makeup. However, its use may lead to skin dryness and enlargement of

pores. Hence, there is a requirement for alternative formulations devoid of alcohol, and the present trend is to employ natural ingredients for this purpose. Recently, the public, particularly among women, favours toners containing natural ingredients due to their organic composition and absence of significant adverse reactions compared to chemical toners (Chasanah, 2019). Cucumbers are a commonly utilized plant in the cosmetic industry due to their high concentration of chemical compounds such as terpenoids, phenolics, flavonoids, and alkaloids. The flavonoid compounds present in cucumber possess antioxidant properties and exhibit the potential to disrupt free radical chains (Agustin & Gunawan, 2019)

The researchers decided on cucumber ingredients due to their lack of alcohol content and high levels of vitamin C, antioxidants, and water. These properties make cucumbers an ideal choice for refreshing facial skin. Another objective of utilizing herbal toners is to enhance efficiency and cost-effectiveness. Cucumbers offer notable benefits, including a remarkable cooling sensation on the skin. Subsequently, these naturally occurring constituents facilitate the cleansing of facial pores by removing surplus sebum and impurities. In addition to its refreshing properties, cucumber toner has demonstrated efficacy in moisturizing the skin and regulating facial oil balance (Chasanah, 2019).

According to a study by (Badriah et al., 2021) entitled "Formulation and Antioxidant Effectiveness Test of Handbody Lotion 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L.)" states that 70% ethanol extract of cucumber fruit has antioxidant activity with an average IC_{50} value of 27.56 µg/ml and enters the category of powerful antioxidants. Based on the background that has been described, this research aims to formulate and carry out antioxidant activity tests preparation of face toner extract from ethanol 70% cucumber (Cucumis sativus L.)

2. METHODS

2.1. Tools

The tools used in this study were a set of glassware, mortar and stamper, analytical balance (ohaus), furnace, karl fischer (Metrohm), moisture balance (Bel engineering), rotary evaporator (IkA), pH meter (Ohaus), water bath, UV-Vis's spectrophotometry (Shimadzu UV-I900i).

2.2. Materials

The materials used in this study were Cucumber (*Cucumis sativus* L.), DPPH, 70% ethanol, glycerine, propylene glycol, nipagin, nipasol, distilled water, and vitamin C. The part of the plant used was the selected green and fresh cucumber (*Cucumis sativus* L.).

2.3. Work Procedures

2.3.1. Plant Determination

Determination of cucumber (*Cucumis sativus* L.) was carried out at Herbarium Bogoriense, Botany Division, Centre for Biological Research and Development, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor KM.46 Cibinong Bogor, 16911-West Java.

2.3.2. Production of Cucumber Simplicia (Cucumis sativus L.)

35 kg of freshly harvested cucumbers (*Cucumis sativus* L.) underwent wet sorting to eliminate extraneous matter, including undesired plant components and other impurities in the sample. Subsequently, the newly acquired samples were subjected to a washing procedure. Then, the cucumbers were sliced and segregated from the seedless part of the contents. After that, the desiccation procedure is executed using an oven set at a temperature range of 40-50^0 C. After the drying stage, a dry sorting procedure is implemented to eliminate extraneous substances that may have been acquired during the aforementioned drying process. The uncontaminated specimens were blended using a blender until they achieved a powdered consistency and subsequently filtered through a No. 40 mesh sieve to ensure uniformity in size. The next step is to measure and document the weight of the simplicia. Subsequently, the simplicia quality parameter examination

was conducted, encompassing distinct parameters such as organoleptic assessments and non-specific parameters such as moisture content, ash content, and drying shrinkage (Heniarti *et al*., 2016).

2.3.3. Preparation of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.)

The extraction process was carried out for 5 days and was divided into 2 parts, the first was the 3-day maceration, and the second was maceration for 2 days. Simplicia was weighed as much as 600 grams, put into a container, and then soaked using 70% ethanol solvent for as much as 6000 ml covered with aluminium foil for 3 days (stirred every day). Then, it was filtered using filter paper and obtained macerate 1 and dregs 1. The dregs were re-soaked using 3000 ml of 70% ethanol solvent for 2 days (stirred every day), then filtered using filter paper and obtained macerate 2 and dregs 2. Furthermore, macerates 1 and 2 are combined into one, and then concentrated using a rotary evaporator until a thick extract is obtained. After that, a parameter test for extract quality consisting of specific parameters, including organoleptic observations, and non-specific parameters, including water content, ash content, and residual solvent was conducted.

2.3.4. Phytochemical Screening

Phytochemical screening was carried out to determine secondary metabolite compounds present in 70% ethanol extract of cucumber (*Cucumis sativus* L.). The Phytochemical screening included:

Alkaloid - The experimental procedure involved crushing a 2 g sample in a mortar, followed by adding 5 mL of 25% ammonia. Subsequently, 20 millilitres of chloroform were introduced into the mortar and subjected to vigorous crushing. Then, the amalgam was subjected to filtration, and the resultant filtrate was subsequently applied onto filter paper, followed by the application of Dragendorff reagent. The observed orange colour indicates the presence of alkaloids in the sample. The residual filtrate underwent two extractions in a separatory funnel utilizing a 10% hydrochloric acid solution. The aqueous layer is distinctively partitioned from the organic layer. The water layer was extracted and subsequently transferred into a test tube for analysis via the Mayer and Dragendorff reactions. A positive outcome was indicated by a white precipitate upon reaction with Mayer's reagent and a red precipitate upon reaction with Dragendorff's reagent (Aprilliani, 2018).

Flavonoid - The experiment involved heating a sample weighing 2 g in 100 mL of water for 15 minutes, followed by filtration to collect the resulting filtrate. A volume of 5 mL filtrate was combined with 0.1 g of magnesium powder, 1 mL of hydrochloric acid, and 5 mL of amyl alcohol. The resulting mixture was agitated and subsequently allowed to undergo phase separation. The development of red, yellow, or orange colour on the amyl alcohol layer is indicative of the presence of flavonoids with positive characteristics (Aprilliani, 2018)

Saponin - 2 g of sample was heated in 100 mL of water for 15 minutes, then filtered, and then the filtrate was collected. 10 mL of the filtrate was put into a test tube. The tube is shaken vigorously for 10 seconds, if foam forms which can last for 1 minute, it is suspected to contain saponins. Then drop the 2 N HCl solution into the test tube. If the foam does not disappear, this indicates that the sample is positive for saponins (Aprilliani, 2018)

Tanin - One gram of the sample was introduced into 100 mL of heated water, subjected to boiling for 15 minutes, and subsequently filtered. The experimental protocol entails the preparation of three test tubes, each of which should be filled with 5 mL of filtrate solution. In this experiment, three tubes were utilized. Tube 1 underwent a reaction with a 1% iron (III) chloride solution, and the presence of polyphenol compounds was confirmed by the formation of blue ink or black-green colour. Gelatine was added to the second tube, and the formation of a white precipitate indicated the presence of tannin. In the third tube, stiasny reagent (30% formaldehyde: HCl 2:1) was added, and the mixture was heated in a 90°C water bath. The

formation of a pink precipitate indicated a positive result for simplicia containing tannins. The precipitate in the third tube was filtered, and the filtrate was mixed with a 1% iron (III) chloride solution. The presence of error tannins was confirmed by the formation of blue ink or greenish-black colour in positive samples (Aprilliani, 2018)

Steroid/Triterpenoid - A total of 1 g of sample was macerated with 20 mL of n-hexane for 2 hours, then filtered and then the filtrate was collected. 5 mL of the filtrate was evaporated in an evaporating cup. The Liebermann-Burchard reagent was added to the evaporated residue. The formation of red to violet colour indicated the presence of triterpenoids in the sample (Aprilliani, 2018).

2.3.5. Formulation of Face Toner of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.)

The formula for face toner preparations comes from (Hilmarni et al., 2022) Utilization of Aromatic Water/Hydrosol of Torbangun Leaves (*Plectranthus Amboinicus* L). In this case the renewal of the formula for the preparation of Face toner can be seen in the following **Table 1**.

Table 1. Formula of 70% ethanol extract face toner cucumber fruit (*Cucumis sativus* L.)

Materials	F0(-)	F1	F2	F3	F4(+)
Cucumber extract	-	0.5	1	1,5	
Nipagin	0.02	0.02	0.02	0.02	Antioxidant
Nipasol	0.02	0.02	0.02	0.02	Toner
Glycerine	10	10	10	10	Brand X
Propylene glycol	10	10	10	10	
Distilled water	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml	

Note:
F0 = Face toner preparation without cucumber fruit extract (*cucumis sativus* L.) 0%
F1 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 0,5%
F2 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 1%
F3 = Face toner preparation of cucumber fruit extract (*cucumis sativus* L.) 1,5%
F4 = Brand X antioxidant toner

Cucumber fruit extract (*Cucumis sativus* L.) was formulated into a liquid preparation for use as a facial toner. Preparation of cucumber fruit extract is performed following the concentration that has been determined, with sufficient amounts of distilled water added beforehand. Nipagin and nipasol are weighed, then dissolved in water and stirred until homogenous. Earlier, glycerine and propylene glycol were measured and added to the solution. Then, cucumber extract was added, followed by the rest of the distilled water, and stirred until uniform. Finally, they were placed in a container.

2.3.6. Physical Evaluation of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L) Face Toner

Organoleptic Test - Organoleptic examination is carried out by visually looking at the physical form, which includes the colour, shape, and smell of the preparation.

Homogeneity Test - Homogeneity testing is carried out by taking 10 ml of the toner formula preparation, then putting the toner into the glass beaker and then observing the arrangement of coarse particles or inhomogeneity in the toner preparation.

pH test - The pH measurement begins with pH meter calibration. Calibration is performed with a buffer solution of pH 4.01 and pH 6.86, then the pH meter is turned on by pressing the on button, the pH meter is inserted into the container containing the toner preparation to be tested, dipped into the water containing the toner preparation, and the scale will move until the numbers stop moving.

Hedonic test - Hedonic test or preference test for face toner preparation is conducted by pouring it on cotton and paying attention to aroma, colour, and texture. A test is performed on the

prepared substance, which is then poured onto cotton or the palm of the hand and applied to the face. Twenty panellists who previously utilized face toner was examined.

2.3.7. Antioxidant Activity Test of Cucumber Fruit Extract (Cucumis sativus L.) With DPPH Methods

Preparation of solution DPPH 0,05 mM - A total of 1.97 mg of DPPH was weighed and then put into a 100 ml volumetric flask added with methanol until the boundary mark was shaken homogeneously, so that a DPPH solution with a concentration of 0.05 mM was obtained (Hasanah *et al*., 2017).

Maximum wavelength determination - Maximum absorption is achieved by determining the longest wavelength. Using a visible spectrophotometer, the absorbance of up to 4 ml of a 0.05 mM DPPH solution in a cuvette was measured at wavelengths between 400 and 800 nm to determine the maximum wavelength. The maximum absorbance value yields the maximum wavelength (Hasanah et al., 2017)

Determination of operating time - Operating time was determined by taking 50 µL of the test solution plus 4.0 ml of 0.05 mM DPPH solution then vertexing and measuring at 0, 5, 10, 15, 20, 25 and 30 minutes at the maximum wavelength obtained from the predetermined wavelength. The minute that produces the most stable absorbance (0.2-0.8) of DPPH free radical immersion is the operating time (Mulangsri *et al*., 2017)

Preparation of blank solutions - 2 ml of DPPH solution (0.05 mM) was put into a test tube and added by 2 ml of methanol p.a., which is then covered with aluminium foil. Then it was homogenized with a vortex and incubated in a dark room for the operating time (Fathurrachman, 2014). Then the absorption is measured at the maximum wavelength from the results of the measurements that have been made.

Preparation of Vitamin C Solution - An accurate weigh of 10 mg of vitamin C powder is dissolved in 100 mL of methanol p.a in a 100 mL volumetric flask to obtain a concentration of 100 ppm (mother liquor). Then from the mother liquor, a concentration series of 1, 2, 3, 4, and 5 ppm was made in a volumetric flask, and the volume was made up with methanol p.a up to 5 mL. Each concentration of 2 mL of vitamin C comparison solution was put into a test tube, and then added by 2 mL of 0.05 mM DPPH solution homogenized with a vortex. Then, it was incubated in a dark room during the operating time (Fathurrachman, 2014). Then, the absorption is measured at the maximum wavelength from the results of the measurements that have been made (Zaky *et al*., 2021)

Solution Preparation and Antioxidant Activity Testing of 70% Ethanol Extract of Cucumber Fruit (Cucumis sativus L.) - 2.5 mg of extract was weighed and dissolved in 10 ml of methanol p.a until homogeneous to obtain a concentration of 250 ppm (mother liquor). The main solution was pipetted as much as 0.08; 0.16; 0.24; 0.32, and 0.4 ml into a 10 ml volumetric flask to obtain a test solution concentration of 2; 4; 6; 8, and 10 ppm. Then, the volume was made up of 10 ml with 2 ml of methanol p.a. for each sample solution put into a test tube, then added by 2 ml of 0.05 mM DPPH solution, homogenized with a vortex. It was then incubated in a dark room during the operating time (Fathurrachman, 2014). Then the absorption is measured at the maximum wavelength from the results of the measurements that have been made.

Preparation of Solutions and Antioxidant Activity Test of Face Toner Preparations - Toner has weighed carefully as much as 10 mg and dissolved in 10 mL of methanol p.a until homogeneous, so that a concentration of 1000 ppm was obtained. This solution was then made into a series of concentrations of 50, 100, 150, 200, and 250 ppm in a 10 ml volumetric flask and the volume was made up with methanol p.a up to 10 ml. 2 ml of each test solution was pipette into a test tube, and 2 ml of the main DPPH solution was added, then homogenized, and measured with UV-Vis's spectrophotometry.

3. RESULT AND DISCUSSION

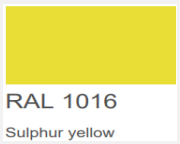
3.1. Plant Determination

The results of the determination carried out at the Herbarium Bogoriense, Botany Division of the Centre for Biological Research and Development-BRIN (National Research and Innovation Agency) Cibinong, showed that the cucumber obtained from Sodong Village RT 002/ RW 003, Tigaraksa sub-district, Tangerang regency, is a cucumber fruit plant with the Latin name Cucumis sativus L in the tribe of Cucurbitaceae.

3.2. Preparation of Cucumber Simplicia

The harvested cucumber (*Cucumis sativus* L.) fruit was subjected to wet sorting to separate the impurities or plant parts not used in the study and collected as much as 35 kg. After that, it was washed with running water. Clean cucumbers are then chopped and separated from the seedless part of the contents to reduce the particle size and increase the surface area. During the extraction process, direct drying is carried out for 7 days using sunlight covered with a black cloth and dried. It used the oven at 50ºC. After drying, dry sorting was carried out and pulverized with a blender to obtain simplicia powder, then sifted using mesh No.40. The simplicia powder was weighed and a yield of 700 g was obtained. After that, a simplicia quality parameter test was carried out to determine the characteristics of the simplicia and ensure that the simplicia used met the specified quality. The results of simplicia quality parameters can be seen in **Table 2.**

Table 2. The results of testing the quality parameters of cucumber (*Cucumis sativus* L.) simplicia

Parameter	Characteristics	Result ± SD	Condition
Non specific	Water content	6.44	<8.5%
	Ash content	5.39 ± 0.37%	<7.2%
	Drying shrinkage	8.0± 0.3%	<10%
Parameter	**Characteristics**	**Condition**	
Specific	Organoleptic:	Powder	
	• Shape		
	• Colour		
	• Aroma	Cucumber typical Bitter	
	• Taste		

3.3. Preparation of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L.)

As much as 700 grams of cucumber simplicia were macerated using 10 L of 70% ethanol for 3x24 hours and stirred every morning and evening. After the maceration process, filtering is carried out to obtain macerate and dregs. The filtered dregs are re-macerated for 2x24 hours. The macerate obtained was concentrated using a rotary evaporator, and then it was concentrated again using a water bath until a thick extract was obtained. The results of the thick extract obtained were 196.23 grams. The yield of the extract obtained was 32.70%. After that, an extract quality parameter test was carried out to determine the characteristics of the extract and ensure that the extract used met the specified quality. The results of simplicia quality parameters can be seen in **Table 3**.

3.4. Phytochemical Screening

Phytochemical screening aims to determine secondary metabolite compounds contained in cucumber rind simplicia and 70% ethanol extract of cucumber fruit. The results of the phytochemical screening can be seen in **Table 4**. The results of the phytochemical screening of 70% ethanol extract of cucumber fruit (Cucumis Sativus L.) revealed that it contains flavonoids, saponins, and alkaloids.

Table 3. The results of testing the quality parameters of 70% ethanol extract of cucumber fruit

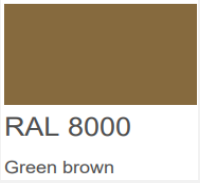
Parameter	Characteristic	Result ± SD	Condition
Non specific	Water content	18.31	<30%
	Ash content	8.15 ± 0,17%	<9.2%
	Residual Solvent	0.89	<1%
Parameter	**Characteristic**	**Condition**	
Specific	Organoleptic: • Shape • Colour • Aroma	Thick  Cucumber typical	

Table 4. Phytochemical screening results

No	Phytochemical Test	Result
1	Flavonoid	+
2	Saponin	+
3	Steroid	-
4	Tanin	-
5	Alkaloid	+

3.5. Physical Evaluation of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L) Face Toner

Physical evaluation of the preparation is a parameter set to determine the stability of the preparation including organoleptic, pH, homogeneity and hedonic tests. Results of dosage formulation Cucumber extract face toner can be seen in **Figure 1**.

Figure 1. Face toner preparation of 70% ethanol extract of cucumber Fruit (*Cucumis sativus* L.)

3.5.1. Organoleptic Test

Organoleptic tests were conducted to determine the physical appearance of the antioxidant toner preparations, including their shape, colour, and odor. The preparations were stored for three weeks at three-week intervals, with various concentrations exhibiting relatively stable colour, shape, and odor stability. Face toner does not undergo any changes during storage. The results of the organoleptic test indicate that the higher the extract concentration in each formula, the more concentrated the colour concentration, which has a significant/visible difference in colour intensity between each formula. Formula 3, with a 1.5% extract concentration, has a darker brown hue than other formulas (Saratiana, 2020).

3.5.2. Homogeneity Test

The homogeneity test is performed to determine whether or not the antioxidant toner ingredients are well mixed. After 3 weeks and a time interval of homogeneity test results on face toner preparations, it was determined that the toner base and the active ingredient were evenly mixed.

3.5.3. pH test

The purpose of the pH test is to determine whether the preparation's pH is compatible with the pH of the skin. A pH meter is used to determine the pH of toner formulations. The test was conducted for three weeks with a weekly observation interval for all formulas. The pH test results are presented in **Table 5**.

Table 5. Face toner pH test results

Formulation	pH Testing, Week -		
	I	II	III
F0 (0%)	6.1	5.9	5.7
F1 (0,5%)	5.5	5.5	5.3
F2 (1%)	5.3	5.2	5.1
F3 (1,5%)	5.0	4.9	4.7

Note:
F0: Formula with concentration without cucumber fruit extract 0 %
F1: Formula with concentrated cucumber fruit extract 0.5 %
F2: Formula with concentrated cucumber fruit extract 1 %
F3: Formula with concentrated cucumber fruit extract 1.5 %

Based on **Table 5** of the pH test, the weekly pH decreases proportionally to the concentration of cucumber fruit extract. This indicates that the pH of cucumber fruit extract (Cucumis sativus L.) is low enough to affect the formulation. In this study, the overall pH of the face toner formulations varied from week 1 to week 3 between 4.7 and 6.1. These results are still within the 4.5-8 pH range recommended by SNI for healthy skin. It will likely not be irritating. In conclusion the face toner containing cucumber fruit extract met the pH requirements for skin preparation.

3.5.4. Hedonic Test

Hedonic test or preference test for face toner preparations is conducted by paying attention to aroma, colour, and texture. Examination was carried out on 20 panellists. The hedonic test results are shown in **Table 6**.

Table 6. Face toner hedonic test results

Sample	Parameter	Dislike	Quite Like	Like	Really Like
F0	Colour	4	5	6	5
	Smell	4	7	7	2
	Texture	4	6	5	5
F1	Colour	0	4	12	5
	Smell	1	5	9	5
	Texture	1	3	10	6
F2	Colour	0	5	11	4
	Smell	2	5	5	8
	Texture	0	5	9	6
F3	Colour	5	3	5	7
	Smell	2	7	9	2
	Texture	1	6	10	3

According to the table, the hedonic test results for face toner preparations obtained in terms of the colour of the formula that panellists liked the most was F1. The preference factor for each

panellist formula that affected it was the addition of cucumber fruit extract. The higher the concentration, the darker the resulting colour. However, with only a small amount of the product, the colour intensity will appear natural and attractive.

Regarding aroma, F1 (Cucumber Fruit Extract Face Toner), the most popular formula among panellists, possesses a distinct cucumber aroma. In the term of texture, the panellists preferred F1 because the resulting texture was soft and not excessively sticky.According to the results of the hedonic test, the preferred formula among the panellists is F1 with a 0.5% extract concentration.

3.6. Ascorbic Acid Antioxidant Activity Test as a Comparison

The use of ascorbic acid as a comparison control in testing antioxidant activity is to find out how strong the antioxidant potential of the extract when compared to ascorbic acid. If the IC50 value of the sample is equal to or close to the IC_{50} value, it can be said that the sample has the potential to be one of the very strong antioxidant alternatives. **Figure 2** reveals that the IC_{50} value of ascorbic acid was 7.768 µg / ml. A very strong antioxidant category, namely IC_{50} values are in the range of 0-50 µg / ml. This suggests that ascorbic acid has a very strong power of antioxidant activity (Zaky *et al*., 2021).

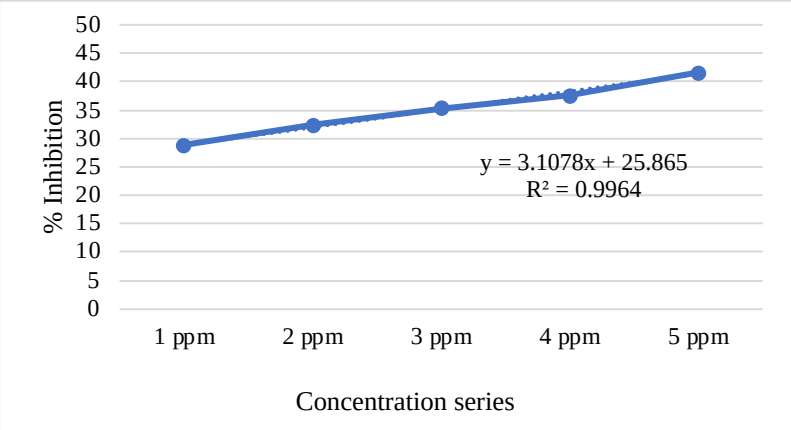


Figure 2. Curve % inhibition of ascorbic acid antioxidant test as a comparison control

3.7. Antioxidant Activity Test of 70% Ethanol Extract of Cucumber Fruit (*Cucumis sativus* L) Face Toner

The face toner preparation was formulated using a concentration of 0.5%, 1% and 1.5% cucumber fruit extract. This concentration was chosen because it was a cosmetic preparation for skin care. Extract antioxidant activity test was carried out to ensure that cucumber fruit extract has antioxidant activity. This is evidenced by the IC_{50} value of cucumber fruit extract of 21.22 µg/ml. The IC_{50} value belongs to the powerful antioxidant category.

The formula was made by increasing the concentration of the extract, starting from a concentration of 0.5; 1; and 1.5%. With an increase in the concentration of the extract in the formula, the greater the % inhibition obtained. For F1 which contained 0.5% cucumber fruit extract, the IC_{50} was 128 µg/ml—in F2, cucumber fruit extract of 1% received IC_{50} of 91.017 µg/ml. Whereas for F3 cucumber fruit extract of 1.5% obtained IC_{50} of 62.218 µg/ml. From the results of tests that have been carried out on formulas that were given extracts, it can be concluded that formulas with extract concentrations of 0.5; 1; and 1.5% still has antioxidant activity and is classified as a strong to moderate antioxidant, where the strong to moderate antioxidant category is 50-150µg/ml. In other words, the less antioxidant effect in the formula, the stronger the antioxidant activity.

Measurement results absorbance and % inhibition of cucumber fruit extract can be seen in **Table 7**.

Table 7. Test results of antioxidant activity of cucumber fruit extract (*Cucumis sativus* L.)

Sample	Concentration (ppm)	Absorbance			
		U 1	U 2	U 3	Mean
Cucumber fruit extract	blank	0.307	0.307	0.307	0.307
	2 ppm	0.283	0.284	0.284	0.284
	4 ppm	0.271	0.271	0.271	0.271
	6 ppm	0.257	0.257	0.257	0.257
	8 ppm	0.243	0.243	0.244	0.243
	10 ppm	0.230	0.230	0.230	0.230

The results show that cucumber fruit extract has antioxidant activity with very strong antioxidants, namely with an average value of IC_{50} 21.22 µg / ml. This is because the active substance used in the preparation, cucumber fruit extract, has compounds that act as antioxidants, namely flavonoids. The content of this secondary metabolite is antioxidant that affect the results of antioxidant activity tests. Based on research by Badriah *et al*. (2021) entitled "Formulation and Test of the Effectiveness of Antioxidant Handbody Lotion Ethanol Extract 70% Cucumber (Cucumis sativus L.)" states that 70% ethanol extract of cucumber fruit has antioxidant activity with an average value of IC_{50} which is 27.56 µg / ml and is included in the category of very strong antioxidants. Measurement results absorbance and % inhibition F3 can be seen in **Table 8**.

Table 8. F3 Antioxidant activity test results (extract 1.5%) of cucumber fruits

Sample	Concentration (ppm)	Absorbance			
		U 1	U 2	U 3	Mean
F3 (extract 1.5%) cucumber fruits	blank	0.540	0.540	0.540	0.540
	50 ppm	0.275	0.275	0.275	0.275
	100 ppm	0.255	0.256	0.256	0.256
	150 ppm	0.239	0.239	0.239	0.239
	200 ppm	0.223	0.223	0.223	0.223
	250 ppm	0.204	0.205	0.205	0.205

The result for F3 toner was determined by IC_{50} value of 62.218 g/ml. This shows that F3 as the highest concentration for facial toner preparations of cucumber fruit extract has relatively high antioxidant activity. To sum up, the greater the concentration of cucumber fruit extract added, the greater the antioxidant activity obtained.

Table 9 indicates the IC_{50} analysis value of ascorbic acid results of 7.768µg / ml. The results show that vitamin C has a very strong antioxidant activity. For the yield of cucumber fruit extract (Cucumis sativus L.) of 21.22µg / ml, it shows that cucumber fruit extract has a very strong antioxidant activity. While for the preparation, it can be seen that the antioxidant activity of the F0 face toner preparation test solution without extract yields an IC_{50} value of 213.72 µg / ml, indicating that F0 without the addition of 70% ethanol extract face toner preparation extract has the strength of antioxidant activity which is relatively weak as a face toner preparation because there are only excipients. F1 face toner preparation with 0.5% extract concentration has an IC_{50} value of 128 µg / ml, indicating that F1 with the addition of 0.5% extract of 70% ethanol extract face toner preparation of cucumber fruit has also weak power of antioxidant activity as a face toner preparation. F2 face toner preparation with a concentration of 1% has an IC_{50} value of 91.017 µg / ml, indicating that F2 with the addition of 1% extract of 70% ethanol extract face toner preparation of cucumber fruit has the power of antioxidant activity which is classified as a strong face toner preparation. Likewise, F3 face toner preparation with a concentration of 1.5% has an IC_{50} value of 62.218 µg / ml, showing that F3 with the addition of 1.5% extract of 70% ethanol extract face toner preparation of cucumber fruit has the power of antioxidant activity which is classified as strong as a face toner preparation. It can be said that the higher the concentration of cucumber fruit extract added, the higher the antioxidant activity obtained. F4 toner preparation brand x has an IC_{50} value of 58.444 µg / ml, this shows that formula 4 brand x

has the power of antioxidant activity which is classified as strong as a positive control toner preparation. research on the effectiveness of handbody lotion preparations had the highest antioxidant activity in the third formula with a concentration of 1.5% at 91.657 ppm. It indicates that toner preparations have higher antioxidant activity than lotions. In conclusion, the higher the concentration of antioxidant activity, the greater the antioxidant activity (Aprilliani *et al*., 2022). Results can be seen in the **Table 9**.

Table 9. Linear regression results and IC_{50} values obtained for Face Toner Preparation 70% Ethanol Extract of Cucumber Fruit

Test Solution	%Inhibition	IC_{50}
Vitamin C	41.666 44.444 47.037 49.074 51.851	7.768
Cucumber fruit extract	41.666 44.444 47.037 49.074 51.851	21.22
F0 (0%)	45.74 48.518 51.296 53.888 57.222	213.72
F1 (0.5%)	45.74 48.518 51.296 53.888 57.222	128
F2 (1%)	47.407 50.925 53.703 56.296 59.444	91.017
F3 (1,5%)	49.074 52.592 55.74 58.703 62.037	62.218
F4Toner Merk X	49.629 52.037 54.259 56.481 58.888	58.444

Based on the results, the face toner preparation in formula 3 with a concentration of 1.5% had a strong IC_{50} value of 62.218 g/ml while the results of the cucumber fruit extract itself had a very strong IC_{50} value of 21.22 g/ml. The difference in these results was due to the fact that the extract has only a small amount as a preparation compared to pure extract. Thus, this is one of the causes for the disparity between the IC_{50} values of face toners and pure extracts.

The statistical data analysis of antioxidant activity test for each formula with the DPPH method was conducted by using the IBM Statistical Product and Service Solution (SPSS) 25.0 application with the One-Way ANOVA method. It aims to determine whether there are significant differences between treatment groups among the five treatment group formulas. The One-Way

ANOVA parameter test has several requirements to meet, including the data obtained must be normally distributed and the variance of the data obtained must be homogeneous.

The normality test results of the One-Sample Kolmogorov-Smirnov Test show that the data is not normally distributed because it has a significance value or p=0.039, meaning that a significant value of p<0.05. Meanwhile, the homogeneity test results showed a p-value or significance of p = 0.062, indicating that the data is homogeneous because of the significance value of p <0.05.

Subsequent analysis used the one-way analysis of variance method to determine whether there is a significant difference in each treatment group. The hypothesis of ANOVA is that the results obtained a significance value of 0.000, indicating that there is a significant difference from each formula due to the p value <0.05. However, since the data is not normal and homogeneous, it is continued using the Kruskal Wallis test. It is to determine whether there is a difference between variables for data that does not meet the requirements of the ANOVA test. The results of the Kruskal-Wallis's test showed a significant difference between the five treatment groups. The Mann-Whitney test was carried out in the next step to find out which group had a significant difference.

The Mann-Whitney test revealed a significant difference between the negative control (F0) and the treatment group that received extracts F1, F2, and F3, with a significance value of less than 0.050, indicating a significant difference. This difference is evident from the fact that the IC_{50} value for F0 is greater than the IC_{50} value for the group that received the extract, indicating that the lower the IC_{50} value, the greater the antioxidant activity.

Further comparisons between extracts F1 and F2, F1 and F3, and F2 and F3 revealed a significance value of less than 0.050, indicating that there was a significant difference. It can be seen in the IC_{50} value obtained for F3, which is smaller than those obtained for F1 and F2 or the smaller the IC_{50} value obtained, the greater the antioxidant activity. To conclude, the extract's antioxidant activity increases with its concentration.

The significance value of the comparison between the positive controls (F4) and the treatment group (F0, F1, F2, F3) is less than 0.050, indicating a significant difference. This distinction is evident from the IC_{50} obtained for F4, which has an IC_{50} value. It is smaller than the treatment group, meaning that the greater the antioxidant activity, the smaller the IC_{50} value. The treatment group has not been able to outperform the positive control group.

4. CONCLUSION

Physical evaluation tests show that it meets the physical requirements of toner (organoleptic, homogeneous, pH, and hedonic tests). Antioxidant activity cucumber fruit extract face toner with concentrations of 0.5%, 1% and 1.5% has IC_{50} values of 128 ppm, 91.017 ppm, and 62.218 ppm respectively. The SPSS analysis reveals a significant difference in the IC_{50} value, by which F3 has a smaller IC_{50} value than other formulas, meaning that the smaller the IC_{50} value, the stronger the antioxidant activity. In conclusion, the higher the concentration of the extract, the stronger the antioxidant activity.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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