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Antioxidant Activity and Irritation Potency of Face Tonic Formulation from Ethanol Fraction of Sappan Wood (*Caesalpinia Sappan* L.)

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ABSTRACT

Sappan wood is a medicinal plant that contains phenolic compounds such as brazilin. It can be used to treat photoaging due to oxidative stress. Face tonic is one type of facial skin care cosmetic that has this purpose. The research aims to determine the formula, percentage inhibition, and irritation potency of face tonic from the ethanol fraction of sappan wood. The ethanol fraction of sappan wood was formulated into a face tonic preparation using glycerin and propylene glycol as cosolvents. The evaluation of face tonic involved physical quality, antioxidant activity using the DPPH method, and the irritation-potential test using the HET-CAM method. The results showed that the two face tonic formulas had good homogeneity, with a pH value of 4.5 and a viscosity of 2.5 cps (FI) and 2.6 cps (FII). Face tonic formulas had inhibitory activity at concentrations of 500 ppm, 550 ppm, 600 ppm, 650 ppm, and 700 ppm, with percentages of inhibition of 21.9%, 22.7%, 23.6%, 24.8%, and 25.3 % (FI), and 20.6%, 21.7%, 22.8%, 23.4%, and 24.1% (FII). The irritation score of each formula was 0 (non-irritant) and 5.44 (moderate irritant). The rise in % inhibition indicates an increase in the antioxidant activity of the two face tonic formulas. Irritation appeared on the FII. FI has the best antioxidant activity, non-irritant, and safe to use.
Keywords: Antioxidant activity, face tonic, irritation potency, sappan wood (*Caesalpinia sappan* L.)

INTRODUCTION

Premature skin aging occurs due to sources of free radicals from the environment, such as air pollution, sunlight, mechanical friction, hot or cold temperatures, and excessive oxidation reactions (Elias *et al*., 2002; Feingold *et al*., 2007). The human body needs antioxidants to prevent these free radical reactions (Sutarna *et al*., 2013). Several studies have been conducted to investigate possible sources that generate antioxidants (Jenie *et al*., 2020). For instance, Huang *et al*., 2020 isolated brazilin compounds from sappan wood (Huang *et al*., 2020). Another previous study found that sappan wood extract was non-toxic (Chu *et al*., 2013), in which the brazilin content of the sappan wood extract reached 8%–22% W/W of the total extract (Hwang & Shim, 2018). Brazilin is the main compound component of sappan wood which has antioxidant activity (Li *et al*., 2013). Antioxidant activity of sappan wood extract in the human skin was shown by the reduction of UVA-induced H_2O_2 production via GPX7 activation. In addition, brazilin exhibits antioxidant effects via glutathione peroxidase 7 (GPX7) (Hwang & Shim, 2018). Brazilin provides a protective effect on UVB-induced loss of fibroblast cell viability. It significantly blocks UVB-induced Reactive Oxygen Species in fibroblasts and inhibits UVB-induced (dose-dependent) expression and secretion of MMP-1/3 (Lee *et al*., 2012). The content of brazilin compounds with antioxidant potential is a solution to overcome skin problems and can be developed in facial skincare products; one of which is a face tonic.

Face tonic is a cosmetic in liquid form. It mainly functions as a refresher and helps to remove dirt and excess oil without drying sensitive skin (Liao & Lien, 2011). This liquid preparation serves as a cleanser and freshener. It is also applied to shrink pores and maintain skin pH. The basic formula of a face tonic is alcohol and water. Preservatives can be added if necessary. The active substance is expected to be dissolved in water or alcohol as a solvent. A solubility in the face tonic

preparation is needed to ensure solubility enhancer (cosolvent) (Schrader & Domsch, 2005). This study used the ethanol fraction of sappan wood as the active ingredient. Because the ethanol fraction is still a crude fraction, it will be difficult to homogenize the face tonic preparations. Thus, it is necessary to add cosolvent (Rowe *et al*., 2009).

In this study, a face tonic formulation from the ethanol fraction of sappan wood was carried out with various cosolvents, glycerin, and propylene glycol, which are potentially useful for facial skincare cosmetics as antioxidants. Glycerin and propylene glycol are used both as a solvent and cosolvent in the cosmetic formulation. Propylene glycol has a minimal irritant in topical preparations, although it is more irritant than glycerin (Rowe *et al*., 2009; Schrader & Domsch, 2005). A face tonic formulation from the ethanol fraction of sappan wood with various cosolvents, glycerin, and propylene glycol can be used as facial skincare cosmetics that are useful as antioxidants. The antioxidant activity of the face tonic is determined using the DPPH method. It measures the percentage of free radical inhibition by antioxidants (Achat *et al*., 2016). The percentage of inhibition is a parameter used to express antioxidant capacity and compare different compounds' antioxidant activity (Chen *et al*., 2013). The method gives rapid results with a colorimetric mechanism using a spectrophotometer (Kandi & Charles, 2019).

A safety test is needed to ensure the safety of face tonic products. The test is called the irritation potency test. Irritation is an inflammatory phenomenon that occurs on the skin after being overly exposed to substances (Yuliani, 2013). In this study, the irritation test employed a qualitative primary irritation test (Bagley *et al*., 1994; Toding & Zulkarnain, 2015) that focused on testing an irritation on the eyes and skin using Hen's egg test–chorioallantoic membrane (HET-CAM) method (Budai *et al*., 2021; Yuliani *et al*., 2016). This method was chosen as it does not depend on the inflammatory mechanism (Öztürk & Kıyan, 2020). The chorioallantoic membrane (CAM) has arteries, veins, and capillaries, so the CAM response to irritants is similar to eye irritation testing in rabbits (Draize method) (Wilson & Steck, 2000). In addition, the irritant assessment score using this method gives similar results to the lactic acid irritation test on human skin (Cazedey *et al*., 2009). However, studies that focus on investigating face tonic formulations are rare to date. Further, those that examine the formulation of face tonic using ethanol fraction of sappan wood are even rarer. Consequently, little information can be obtained regarding the potential irritation of the formula. Therefore, this study aims to make a face tonic formulation and determine the antioxidant activity and irritation potency of the face tonic formula from the ethanol fraction of sappan wood with various cosolvents: glycerin and propylene glycol.

MATERIALS AND METHODS

This study used several materials, such as sappan wood were obtained from Sungai Pangkalan, Bengkayang Regency, West Kalimantan. 96% technical ethanol, technical n-hexane, technical chloroform, glycerin, propylene glycol, sodium benzoate, lactic acid, distilled water, citrus oil, and butylhydroxytoluene (BHT) were purchased from Alkamid Co. (St. Tehran, Iran). Other materials included quercetin and 1,1-diphenyl-2- picrylhydrazyl (DPPH), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), 0.9% NaCl were obtained from PT. Sanbe Farma (Jakarta, Indonesia). The test animals were leghorn chicken eggs.

Sample collection and processing

Sappan wood was obtained from Sungai Pangkalan, Bengkayang district, West Kalimantan, Indonesia. Sappan wood was wet sorted to separate dirt or materials. The wood was then dried using an oven (Memmert UN 55 53L) at 40°C to obtain dry simplicial with a moisture content of 6.43%. The dried sappan wood was then chopped and mashed using a blender (Miyako®) to obtain coarse sappan wood powder. The aim is to reduce the simplicial particle size to expand the simplicia's contact with the solvent during the extraction process. Sappan wood was macerated in a maceration vessel using 96% ethanol and extracted in liquid form using n-hexane and ethanol (1:1). The extract's color of the brazillin pigment contained in sappan wood will produce a fairly sharp color, orange-red to brownish, and stable (Padmaningrum *et al*., 2012). The ethanol fraction was concentrated using a rotary evaporator (Dragon LAB RE-10 Pro) to derive a thick ethanol fraction (FEtOH) (Sari, *et al*., 2021).

Formulation and evaluation of face tonic

Face tonic formulations were based on the basic formula and pre-formulation studies (Table I). The face tonic was made by dissolving the ethanol fraction of sappan wood in propylene glycol (FI) and glycerin (FII).

Table I. Face tonic formula from the ethanol fraction of sappan wood

Composition	Formula (%)		Range Concentration (%)	Use
	FI	FII		
Ethanol fraction of sappan wood	0.02	0.02	-	Active substance
Lactic acid	0.2	0.2	0.015-6.6	Acidulant; skin conditioning
Propylene glycol	7	-	5-80	Cosolvent
Glycerin	-	10	≤50	Cosolvent
Butylhydroksytoluene (BHT)	0.1	0.1	0.0075-0.1	Antioxidant
Sodium benzoate	0.2	0.2	0.1-0.5	Preservative
Ethanol (96%)	5	5	until 30	Solvent
Citrus oil	0.1	0.1	Qs	Perfume
Aquadest	until 100	until 100	until 100	Solvent

Next, ethanol was added and followed by BHT, which had been dissolved in ethanol (ethanol phase). After that, sodium benzoate was dissolved in distilled water and then was added with lactic acid, which was dissolved with distilled water (water phase). The water phase was mixed into the ethanol phase, then stirred until homogeneous. After that, the face tonic was added with citrus oil and then filtered and packaged (Schrader & Domsch, 2005).

The evaluation of the face tonic involved the organoleptic test (color, odor, and texture) (Badan Standarisasi Nasional, 1998), pH value test using pH-meter (PH-108 ATC) (Hasanah & Novian, 2020), homogeneity test, and viscosity test using Brookfield viscometer (Ntech®) (Akib *et al*., 2016). Inclusion categories for organoleptic tests are interested in sensory organoleptic testing and willing to participate; consistent in making decisions; in good health, free from ENT diseases, not color blind and psychological disorders; did not averse to the food to be tested (not allergic); did not do the test 1 hour after eating; wait at least 20 minutes after smoking, eating chewing gum, food and soft drinks; did not test when sick with influenza and eye pain; did not eat very spicy food at lunch, if the test is carried out during the day; did not use cosmetics such as perfume and lipstick and wash hands with odorless soap during the odor test (Badan Standarisasi Nasional, 1998).

Antioxidant activity assay

The antioxidant activity test using the DPPH method was measured according to the method expressed by Yim *et al*. (2019), with some modifications.

Preparation of DPPH solution and Maximum wavelength screening of DPPH.

DPPH solution (50 ppm) is made by weighing 5 mg of DPPH dissolved with 100 mL of absolute methanol. The 4 mL DPPH was incubated for 30 minutes at 37°C in a dark room. The absorbance was determined and the calibration graph was construed.

Preparation of sample solutions

Each face tonic formula (50 mg) was dissolved with absolute methanol to obtain 50 mL of solutions. Then stirred using a magnetic stirrer (SH-2 Digital Lab Thermostatic Hot Plate Magnetic Stirrer Mixer) at a speed of 300 rpm (1000 ppm), diluted into 500 ppm, 550 ppm, 600 ppm, 650 ppm, and 700 ppm.

Preparation of quercetin solution

The 5 mg of quercetin was dissolved with absolute methanol to obtain 10 mL of solutions. Then stirred using a magnetic stirrer at a speed of 300 rpm (500 ppm), diluted into 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

Determination of antioxidant activity of quercetin

The 0.5 mL of each concentration of quercetin then added with 3.5 mL of DPPH. Incubated for 30 minutes at room temperature. The absorbance was determined using UV-Visible spectrophotometer at λmax=516 nm. The calibration graph was construed.

Determination of antioxidant activity of face tonic formulations

The 50 ppm DPPH (in methanol) solution (3.5 mL) was added to 0.5 mL of the sample. And then incubated using incubator (Gemmyco Digital) at room temperature in a dark condition for 24h.

The assessment of absorbance was performed at 516 nm using a spectrophotometer UV-Visible (Shimadzu UV-2600). The calculation of DPPH radical scavenging activity was accomplished by the percentage difference (%) between the absorbance of the sample and control (Yim *et al*., 2019).

$$\% \text{ inhibition} = \frac{\text{control abs} - \text{sample abs}}{\text{control abs}} \times 100\% \quad (1)$$

The increase in % inhibition showed an increase in antioxidant activity (Tsai & Lin, 2019).

Irritation potency test of face tonic with the Hen's Egg Test Chorioallantoic Membrane (HET-CAM) method

The test was measured according to the method stated by Cazedey *et al*. (2009) with modifications. Fertilized leghorn eggs were obtained from a local supplier (Desa Terusan, Mempawah district, West Kalimantan, Indonesia). The eggs were put in an incubator at 37°C and rotated for 10 days. On the last day, the eggs were checked to see the presence of live embryos; then, the egg cavity was marked. After that, the outer shell was cut out using sterile scissors. Previously, the shell was softened with a sterile 0.9% NaCl solution. Then, the egg's outer membrane was moistened with warm 0.9% NaCl solution and put back into the incubator for 5-20 minutes. After the outer membrane was removed, the eggs were sorted without CAM damage. A total of 300 mg of the sample was injected into the CAM, allowed to stand for 20 seconds. After 20 seconds, the CAM was immediately cleaned using sterile 0.9% NaCl. Observations were made for 300 seconds starting after CAM injection. Experiments were carried out on positive control (lactic acid), negative control (sterile water), and both face tonic formulas (Cazedey *et al*., 2009). The data obtained in the HET-CAM test was calculated using the following formula:

$$T = \frac{301-H}{300} \times 5 + \frac{301-L}{300} \times 7 + \frac{301-C}{300} \times 9 \quad (2)$$

Where H denotes the time the hemorrhagic reaction started, L is the time of onset of vascular lysis in CAM and C is the time of onset of coagulation in CAM (Vinardell & Macián, 1994). The results obtained from the calculations were then matched with the values in HET-CAM Irritation Categories (Table II).

Table II. HET-CAM Irritation Categories

HET-CAM irritation score	Irritation category
≤0.9	Non-irritant
1.0 – 4.9	Weak irritant
5.0 – 8.9	Moderate irritant
9.0 – 21	Severe irritant

(Vinardell & Macián, 1994)

The eggs used in the study were immediately destroyed at the end of each test by placing them in a freezer (Wilson & Steck, 2000).

RESULTS AND DISCUSSION

The study was conducted in 4 (four) stages: preparation of samples, formulation of the two face tonic formulas, the antioxidant activity test of the face tonic using the DPPH method, and the irritation potency test using the HET-CAM method.

Face tonic formulation

The face tonic was made into two formulas, using the ethanol fraction of sappan wood with variations of cosolvent: FI (glycerin) and FII (propylene glycol). From each formula, 3 replications were carried out to minimize the error factor during the manufacturing process. The additives used covered propylene glycol and glycerin as cosolvents to increase the solubility of the sample (ethanol fraction) and as humectants. Other additives were lactic acid which served as an acidulant and a skin conditioner, BHT, which functioned as an antioxidant to prevent oxidation of the sappan wood fraction, sodium benzoate as a preservative, citrus oil as a perfume, and ethanol and water as solvents (Rowe *et al*., 2009). The resulting face tonic preparations were evaluated to examine their physical quality, including organoleptic, homogeneity, pH, and viscosity tests (Sari, Widyasari, & Artari, 2021a).

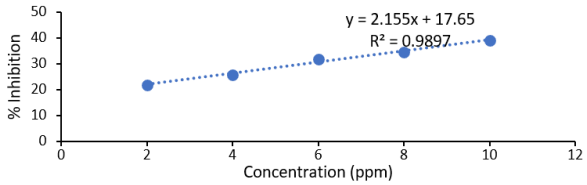
Organoleptic Test

Both formulas showed yellow-orange color and aromatic odor with a clear solution texture. According to some scholars, the colors of the two formulas are influenced by the natural color of brazilin (Girdthep *et al*., 2018) and its acidity level (Jimtaisong & Sarakonsri, 2019; Rina *et al*., 2016).

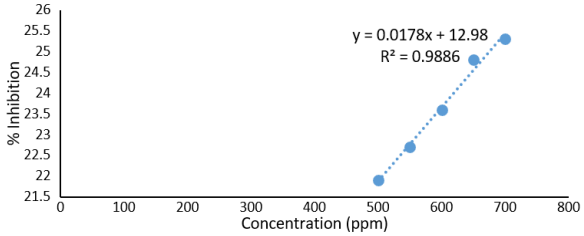
pH Tests

The pH test aims to determine the acidity and basicity levels of the face tonic. The pH standard for face tonic according to SNI number 16.4955.1998 is 3.0-7.0.

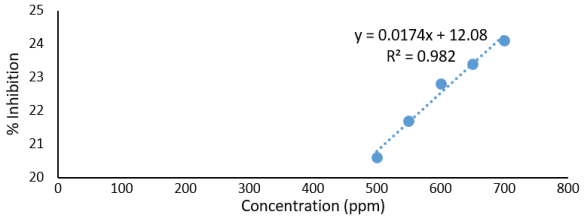
The relationship between quercetin concentration and % inhibition



Relationship between FI concentration and % inhibition



Relationship between FII concentration and % inhibition



Description:
FI: face tonic (with glycerine as cosolvent); FII: face tonic (with propylene glycol as cosolvent)

Figure 1. The curve of the relationship between the concentration of quercetin and face tonic FI and FII to % inhibition

The results of the two formulas showed a pH of 4.5. In other words, all formulas fulfill the pH standard of the face tonic

Homogeneity Test

Both formulas were tested for homogeneity visually (Desriani *et al*., 2018). The homogeneity test was conducted to observe the presence or absence of not homogeneously mixed particles in the preparation. The results showed that the two formulas were homogeneous.

Viscosity Test

Viscosity measurements were carried out using a Brookfield Viscometer. Both face tonic formulas were tested using spindle 2 at a speed of 60 rpm. The face tonic viscosity standard according to SNI number 16.4955.1998 is below 5 cps. Both face tonic formulas have a viscosity of 2.5 and cps, respectively. Therefore, they fulfill the viscosity requirements (Akib *et al*., 2016).

Antioxidant activity test

The linear calibration curve (Figure 1) demonstrated linear responses that confirmed the relationship between concentration and absorbance (Kandi & Charles, 2019). Antioxidant activity by the DPPH method was expressed by % inhibition, which is obtained from the difference in absorption between the absorbance of DPPH and the absorbance of the sample as measured by a UV-Vis spectrophotometer (Sheth & Subrata De, 2012). The increase in % inhibition showed an increase in antioxidant activity.

The antioxidant activity increase of the two face tonic formulas as indicated by the rise in % inhibition (Table III). A quantitative antioxidant activity tested using the DPPH method is widely used in alcohol solutions to estimate the antioxidant capacity of phenolic compounds through their ability to donate H-atoms and/or electrons (Achat *et al*., 2016). DPPH method used to obtain radical inhibition DPPH (Chen *et al*., 2013; Dawidowicz *et al*., 2012). DPPH method based on scavenging effects on DPPH radicals (measures the decrease in DPPH radical absorption after exposure to radical scavengers) (Barreira *et al*., 2013). When the % inhibition is higher, the antioxidant activity is higher (Parsa & Salout, 2016). The antioxidant activity test of face tonic preparations was carried out at a wavelength of 516 nm, the maximum wavelength of DPPH with a DPPH concentration of 50 ppm. To determine the % inhibition, we chose five different concentrations for standard quercetin and the sample, i.e., face tonic formula (Handayani *et al*., 2014).

Table III. Inhibitoty activity test results.

Sample	Concentration (ppm)	Inhibition (%)
Quercetin	2	21.8
	4	25.8
	6	31.8
	8	34.5
	10	39
F I	500	21.9
	550	22.7
	600	23.6
	650	24.8
	700	25.3
F II	500	20.6
	550	21.7
	600	22.8
	650	23.4
	700	24.1

The three test samples, namely quercetin as a reference and the two face tonic formulas, quercetin has the highest absorption percentage, followed successively from the largest to the smallest, namely quercetin, FI (face tonic with propylene glycol cosolvent) and FII (face tonic with glycerin cosolvent) (Table III). The high percentage of quercetin absorption indicates that quercetin has a greater radical scavenging ability than the two face tonic formulas. FI has a greater radical scavenging ability than FII. The rise in % inhibition indicates an increase in the antioxidant activity of the two face tonic formulas. The results show that the two face tonic formulas have inhibitory activity (Handayani *et al*., 2014). The antioxidant activity of the face tonic formula comes from the combined effect of brazilin and other compounds such as flavonoids and other phenolic compounds (Wetwitayaklung *et al*., 2005). Additionally, the presence of brazilin compounds in the chemical composition of the sappan wood extract exhibited antioxidant activities crucial for pharmaceutical uses (Krongrawa *et al*., 2018).

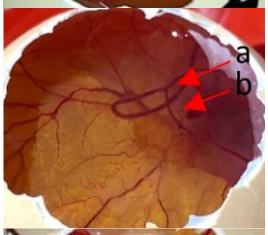
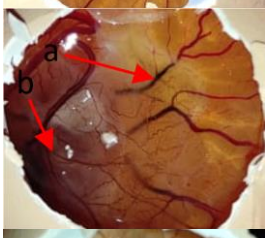
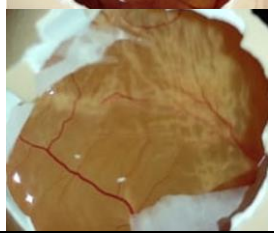
Identification of the antioxidant activity of sappan wood has been carried out in previous studies using the DPPH method, on extract samples and medicinal preparations containing sappan wood extract, where the resulting IC_{50} value was ethanol extract of sappan wood had an IC_{50} value of 101.8 ppm (strong antioxidant activity) (Setiawan *et al*., 2018). There were differences in antioxidant activity between extracts and face tonic products from sappan wood. Based on Mustarichie and Priambodo (2019), in solid dosage formulations, the formula orally disintegrating sappan wood tablets, with a dose of 100 mg/tablet (with a concentration of 0.1%) have antioxidant activity with strong category IC_{50} value of 3.614 ppm, 3.464 ppm, and s 3.173 ppm, respectively (Mustarichie & Priambodo, 2019). Another study tested the IC_{50} value of the solution preparation, hair tonic from ethanol extract, ethanol fraction, and chloroform-methanol fraction of sappan wood (with 0.1% concentration) of 700.859 ppm, 505.169, and 855.930 ppm (no antioxidant activity), respectively (Sari, Widyasari, & Artari, 2021b). There were differences in the results of antioxidant activity in different formulation form. Further research can conduct preformulation studies on the concentration of the sappan wood fraction used to increase its antioxidant activity.

Irritation Potency using Hen's Egg Test Chorioallantoic Membrane Method

The HET-CAM method is used as an alternative method for testing ocular irritation by assessing the potential irritation to the conjunctiva. It responds to irritants by giving an inflammatory reaction similar to conjunctival tissue. Thus, it can be used as an alternative assessment of eye irritation test using rabbit (Draize test) (Bagley *et al*., 1994; Budai *et al*., 2021). The HET-CAM test is used to identify irritation potency for non-irritating or slightly irritating materials, both as raw materials and in product form (Derouiche & Abdennour, 2017). HET-CAM allows us to evaluate the test substance's potential to damage blood vessels that cause bleeding, coagulation, hyperemia, or lysis (McKenzie *et al*., 2015). HET-CAM results are quite good for developing cosmetic products in vitro studies, with an international validation value of 70% (Steiling *et al*., 1999). This method was developed to assess the potential of cosmetic ingredients and products irritation that is applied to the facial skin area near the eyes (Budai *et al*., 2021; Steiling *et al*., 1999). Because a face tonic is applied on the face, it is necessary to test the irritation potential of this product to ensure its safety.

Results of the HET-CAM test on the test sample (Table IV), HET-CAM test has been carried out to determine a test substance's ocular tolerance, toxicity, irritation, and inflammatory potential (Öztürk & Kıyan, 2020). Parameters of the observations were conducted by observing the time of occurrence of hemorrhage, lysis, and coagulation after 300 minutes of giving the test sample (face tonic FI and FII, positive control, and negative control) (Gilleron *et al*., 1996, 1997).

Table IV. Irritation Test Results of Face Tonic Formula Using HET-CAM Method

Sample	Results	HET-CAM Irritation Score	Irritation Category
FI		0	Non-irritant
FII		5.4	Moderate-irritant
K+		9.51	Severe irritant
K-		0	Non-irritant

FI: face tonic (with glycerine as cosolvent); FII: face tonic (with propylene glycol as cosolvent); K+: positive control (lactic acid); K-: negative control (0.9 % NaCl); a: hemorrhagic; b: lysis.

The results of CAM observations on the test sample showed hemorrhage at 40 seconds and lysis at 80 seconds, without coagulation. The irritation score was 9.51 (classified as severe irritation) in the positive control (lactic acid), in the negative control (NaCl 0.9%) and FI with an irritation score of 0 (zero) (non-irritating category), and FII with an irritation score of 5.4 (moderate irritation category). HET-CAM is a method used to classify a compound's irritant potential: irritant (weak, moderate, and severe irritant) or non-irritant (Yuliani *et al*., 2016). FII acted as a moderate irritant by inducing haemorrhage and producing intense vasoconstriction of the CAM vessels (Vinardell & Macián, 1994). FI acted as non-irritant and safe to use.

The ingredients in the face tonic formula that are classified as non-irritant are BHT, citrus oil, and sodium benzoate. Meanwhile, materials that are irritating when used in high concentrations are ethanol and lactic acid. In topical preparations, such as face tonics, propylene glycol provides minimal irritation but is more irritating than glycerin. This aspect underlies the difference in the irritation category between face tonic with glycerin as cosolvent and face tonic with propylene glycol as cosolvent (Rowe *et al*., 2009). In general, HET-CAM test results can be used as a screening for a material or product (Vinardell & Macián, 1994). In FII of face tonic, the category of moderate irritation is included in the category of irritation. Budai, *et al* (2021) categorizes the HET-CAM test scores into three categories, non-irritating (0-0.9), irritating (1-8.9), and severe irritating (9-21) (Budai *et al*., 2021). From these data, the irritation score on the FII indicates that the facial tonic formula is not safe

to use. The results showed that the face tonic formula from the ethanol fraction of sappan wood with glycerin as a cosolvent in FI is safe to use.

CONCLUSION

This study concluded that the two face tonic formulas had good homogeneity, with a pH value of 4.5 and a viscosity of 2.5 cps (FI) and 2.6 cps (FII). The irritation score of each formula was 0 (non-irritant) and 5.44 (moderate irritant). Face tonic formulas had inhibitory activity at concentrations of 500 ppm, 550 ppm, 600 ppm, 650 ppm, and 700 ppm, with percentages of inhibition of 21.9%, 22.7%, 23.6%, 24.8%, and 25.3 % (FI), and 20.6%, 21.7%, 22.8%, 23.4%, and 24.1% (FII). The rise in % inhibition indicates an increase in the antioxidant activity of the two face tonic formulas. Irritation appeared on the FII. FI is non-irritant and safe to use. Irritation appeared on the FII. FI has the best antioxidant activity, non-irritant, and safe to use.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

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