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Antioxidant activity and antiaging gel formulation grapefruit peel (*Citrus maxima* Merr.) ethanolic extract

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ABSTRACT

This study aims to conduct the antioxidant activity test of grapefruit peel ethanolic extracts and gel formulation. Grapefruit (*Citrus maxima* Merr.) is a plant of the Rutaceae family, which has been known to contain phenolic compounds (flavonoids and tannins). Grapefruit skin was very thick (>30% of the total weight of the fruit) and always considered as waste that has not been utilized properly. Phytochemical screening and water content assay was conducted on the simplicia powder. The simplicia powder was extracted by the maceration method with ethanol as the solvent. Dried extract was obtained by evaporating the solvent with a rotary evaporator, then freeze drying. Antioxidant activity test of grapefruit peel ethanolic extracts using free radical trapping DPPH (1,1-diphenyl-2-picrylhydrazil) was measured at a wavelength of 516 nm after 60 minutes at room temperature with vitamin C as a comparison. Preparation of ethanol extract gel formulations skin grapefruit gel base with CMC-Na and was conducted the evaluation of the material of the gel and the activity of anti aging. Results of phytochemical screening showed simplicia powder contains alkaloids, flavonoids, glycosides, saponins, tannins, and steroids. Water content assay results obtained 7.32%. The test results of antioxidant activity in the DPPH free radical trapping obtained the inhibitory concentration 50 (IC₅₀) values 60.182 ppm and vitamin C 2.679 ppm. Preparation of ethanol extract gel grapefruit skin preparation gels that is meets the requirements of its homogeneity, pH, stability, irritation, and testing activities, namely anti-aging moisture, smoothness, large pores, blemishes, wrinkles, wrinkle depth. Grapefruit peel ethanolic extract had a strong category antioxidant activity and can be formulated as material of topical anti-aging gel.

Keywords: Topical gel, anti-aging, antioxidant, DPPH, *Citrus maxima*

INTRODUCTION

Aging is a normal process that occurs in every human being, but it will be a problem if it happens faster than the time or commonly called premature aging. Signs of premature aging can occur in all organs of the human body and the most visible is on the skin. The symptoms can be characterized by the presence of wrinkles, hyperpigmentation and spots on the skin [16,22]. Basically, skin aging is divided into two major processes, such as: chronology aging or intrinsic aging and photoaging [42]. Chronology Aging indicated by changes in structure and function, as well as skin metabolic with age. This process may include dry skin, thin skin, appearance of smooth wrinkles, and skin pigmentation [8,9]. Photoaging is a process that involves the reduction of collagen and elastin fibers of the skin due to UV light exposure [43]. Excessive UV exposure will cause skin damage due to the proteolysis enzyme emergence of free radicals are formed [35,41]. Furthermore, this enzyme will break down collagen, which is located in the dermis [13,29]. Anti-aging is a process which is useful for preventing and slowing the effects of aging in order to help anyone live longer, healthier, and happier [26,28]. Premature aging can be prevented by avoiding free radicals, use sunscreen, consume water, do not smoke, do not consume alcohol, adequate rest and avoid stress.

Oxidative stress is the most important cause in some cases of the disease that often develops today such as cancer, diabetes mellitus, atherosclerosis, cardiovascular disease, premature aging and inflammation that is the result of an imbalance between the formation and neutralization of prooxidant. Oxidative stress is characterized by the emergence of the first free radicals that seek stability by taking a pair of electrons contained in biological macromolecules, such as proteins, lipids and DNA contained in human cells healthy so causing proteins and DNA is damaged [34]. The compounds that can protect human cells from free radicals, among others tocopherol, ascorbic acid and glutathione [33]. Antioxidant chemical compounds derived from plants have a great potential in solving the imbalance between oxidative stress with some degenerative diseases are growing rapidly at this time, because it is necessary a plant chemical compounds that have potent antioxidant properties but cytotoxic weak [33,34]. Many of the chemical compounds of plants that contain compounds called polyphenols have an effect as a catcher of free radicals, including flavonoids, polyphenols, tannins and phenolic terpenes.

Though various citrus species have been the subjects of several researches concerning antiaging activity, little or no work at all has been done on determining the chemical composition and the antiaging potential of pummelo (*Citrus maxima* (Burm.) Merr.), in particular the inedible fruit parts usually just cast aside [6]. Pummelo is a common citrus species in the country, thought to be the local version of grapefruit. In view of previous findings, ascertaining antioxidant activities of a number of phytochemicals inherent in plant food like citrus and the fact that all citrus have similar complex structure regardless of cultivars, pummelo can therefore be a potential replacement for synthetic preservatives therefore providing multiple benefits to consumers by way of furthering its possible usage in the fields of medicine, therapeutics and food technology.

Our skin needs a protective on the outside of the body to overcome the exposure to motor vehicle fumes and ultraviolet rays [1,3]. Protective antioxidants in the form of grapefruit peel extracts that have a high content of flavonoids, namely limonene, naringenin, hesperetin and vitamin C [31,32]. Protection of the skin can be a topical preparation that is applied to the skin to reduce wrinkles and black spots caused by free radicals. The gel is a semisolid material clear, opaque and contains the active substance, is a colloidal dispersion to have the power network caused by interconnected bind on the dispersed phase. Gel-forming substances used as a binder in protective colloids, granulation in suspension, and thickener for oral preparations [7,18]. Gel preparations widely much used in medicinal products and cosmetics also in some industrial processes. Gel preparations are generally made of HPMC (Hydroxy Propyl Methyl Cellulose) propilenglikol or CMC (Carboxy Methyl Cellulose) with the addition of a preservative like methyl paraben [4,5].

MATERIAL AND METHODS

Material

This research was conducted in the Formulation Technology Laboratory and Research Laboratory, Faculty of Pharmacy, University of Sumatera Utara. The tools used in this research are the skin analyzer dan moisture check (Aramo Huvis), analytical balance (Sartorius), and spectrophotometer UV/Vis (Shimadzu). The materials used in this research are methyl paraben, sodium pyrosulfit and glycerine from Merck and grapefruit. Grapefruit obtained from a grapefruit farmer in Matangglumpangdua Village, Bireuen District, Nanggroe Aceh Darussalam Province. Determination of grapefruit was done in Herbarium Medanese, University of Sumatera Utara and gives the following systematic:

Kingdom	: Plantae
Divisi	: Spermatophyta
Class	: Dicotyledoneae
Ordo	: Rutales
Genus	: Citrus
Species	: <i>Citrus maxima</i> Merr.

Methods

Plant Preparation

Grapefruit as much as 10 kg washed and grapefruit peel are collected, cleaned, drained, then cut longitudinally. The skin is then dried in the drying cabinet at a temperature of $\pm 60^{\circ}\text{C}$ until dry, and then stored in plastic bags to prevent the effects of moisture [10,17].

Water Content

The water content assay was conducted using azeotropi (toluene distillation) method. A total of 200 ml of toluene and 2 ml of distilled water, put in a round-bottom flask, fitted the apparatus, then distilled for 2 hours. Distillation was stopped and allowed to cool for 30 minutes, then the volume of water in the receiver tube is read. Put 5 g

simplicia powder that has been weighed carefully, the flask is heated gently for 15 minutes, after the toluene boiling, the drops speed set to be 2 drops per second until most of the water has been distilled, then the speed of distillation is increased to 4 drops per second, after all of the water has been distilled, the inside of the cooler is rinsed with toluene. Distillation is continued for 5 minutes, then the receiver tube is allowed to cool at room temperature. After water and toluene has been separated, the water volume is read to the nearest 0.05 mL. The volume difference of the water that is read in accordance with the water content contained in the material being examined. The water content was calculated in percent [2,10].

Phytochemical Screening

Phytochemical screening carried out on grapefruit peel simplicia powder includes examining chemical compounds alkaloids, flavonoids, glycosides, anthraquinone glycosides, saponins; tannins, triterpenoids and steroids [39,44].

Extraction

Preparation of grapefruit peel ethanolic extract is done by maceration. A total of 200 g of powder simplicia put into maceration bottles, covered with 75 parts of ethanol, closed and left for 5 days protected from light and stirring often. Then filtered, residue washed with ethanol to obtain 100 parts, macerate transferred into a closed vessel, left in place the cool, protected from light for 2 days, then filtered. Macerate obtained and then evaporated at low temperature and pressure so that obtained the dried extract [12,21].

Antioxidant Activity Test

The ability of the grapefruit peel ethanolic extract in reducing DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical oxidation process in methanol solution (resulting in a DPPH color change from purple to yellow). The inhibitory concentration 50 (IC_{50}) values (concentration of samples that can reduce 50% of free radicals) were used as a parameter to determine the antioxidant activity of the test sample [25,27].

a. Preparation of DPPH solution

A total of 10 mg of DPPH was weighed and then dissolved with methanol in a 50 mL volumetric flask to obtain a solution of 0.5 mM DPPH (concentration 200 μg / mL). Then pipette 5 mL of 0.5 mM DPPH, diluted in a 25 mL volumetric flask, obtained DPPH solution (concentration 40 μg / mL) [27,38].

b. Preparation of test sample solution

A total of 25 mg of grapefruit peel ethanolic extract was weighed and then diluted with methanol in a 25 mL volumetric flask to obtain a sample stock solution (concentration 1000 μg / mL). The concentration determined after multiple orientations. The sample stock solution sample pipette 1.0 mL; 1.5 mL; 2.0 mL; 2.5 mL into each 25 mL volumetric flask to obtain the concentration of the test solution 40 μg / mL, 60 μg / mL, 80 μg / mL and 100 μg / mL, into each volumetric flask added 5 mL of DPPH 0.5 mM (concentration 200 μg / mL) and diluted with methanol. Let stand in a dark place for 60 minutes, then absorbance was measured using UV-visible spectrophotometer at a wavelength of 516 nm [14].

c. Making vitamin C solution

A total of 25 mg of vitamin C powder was weighed and then diluted with methanol in a 250 mL volumetric flask to obtain a vitamin C stock solution (concentration 100 μg / mL). The vitamin C stock solution pipette 0.25 mL, 0.50 mL; 0.75 mL; 1.00 mL into each 25 mL volumetric flask to obtain the concentration of the test solution 1 μg / mL, 2 μg / mL, 3 μg / mL and 4 μg / mL, into each volumetric flask added 5 mL of DPPH 0.5 mM (concentration 200 μg / mL) and diluted with methanol. Let stand in a dark place for 60 minutes, then absorbance was measured using UV-visible spectrophotometer at a wavelength of 516 nm.

d. Analysis of Free Radicals Trapping Percent

Determination of free radical trapping percent count with the following formula [27,39].

$$\text{Free radical trapping activity (\%)} = (A_c - A_s) / A_c \times 100\%$$

Description: A_c = Absorbance of control; A_s = Absorbance of the sample

e. Analysis of Inhibitory Concentration 50 (IC_{50})

Calculations used in the determination of free radical trapping activity are inhibitory concentration 50 (IC_{50}), these values describe the concentration of test compounds that can trap free radicals by 50%. The result of the calculation is inserted into the regression equation with the concentration of the sample (μg / mL) as the x-axis and the value of % entrapment (antioxidants) as the y-axis. Specifically, a compound classified to be a very strong if the IC_{50} value

less than 50 $\mu\text{g} / \text{mL}$, strong if the IC_{50} value between 50-100 $\mu\text{g} / \text{mL}$, medium if the IC_{50} value between 101-150 $\mu\text{g} / \text{mL}$ and weak if the IC_{50} value more than 150 $\mu\text{g} / \text{mL}$.

Gel Formulation

Preparations were made based on the standard formula of CMC-Na gel base is as follows [11,23,30]

R/ CMC-Na 5 g
Glycerin 10 g
Propilenglicol 5 g
Aquadest ad 100 ml

Modification to the standard formula is as follows:

R/ CMC-Na 2,5 g
Extract x
Propilenglicol 2,5 g
Aquadest ad 100 ml

On this, the modified formula glycerin formula gel preparations kicked off and replaced with ethanol extracts of grapefruit peel, as glycerin can serve as a skin moisturizer. Weight of CMC-Na also reduced to 2.5% since the concentrations of CMC-Na 5% obtained a very thick gel mass. Anti-aging formula gel preparations with the variation of concentration of ethanol extracts of grapefruit skin made with four formulas like (Table 1) as follows.

Table 1: Formula gel preparations with the variation of concentration of ethanol extracts of grapefruit peel

Ingredients (%)	Formulas			
	A	B	C	D
Extract	-	1	1,5	2
Glycerin	10	-	-	-
CMC-Na	2,5	2,5	2,5	2,5
Propilenglicol	2,5	2,5	2,5	2,5
Aquadest ad	100	100	100	100

Description: Formula A: Blanko (gel without extract)

Formula B: Concentration of ethanol extracts of grapefruit peel 1%

Formula C: Concentration of ethanol extracts of grapefruit peel 1.5%

Formula D: Concentration of ethanol extracts of grapefruit peel 2%

Materials were weighed in accordance with the weighting of each formula into hot porcelain mortar filled with 20 ml of hot water boiling, and newly added CMC-Na while crushing, until a transparent mass is formed. Furthermore added Glycerin (ethanol extracts or replaced grapefruit skin), propilenglicol, methyl paraben and dissolved in 5 ml of water, so that the obtained gel preparations with an overall weight of each formula 100 g, and stir until homogeneous [11,37].

Homogeneity

Examination methods: Observations of homogeneity each formula were done by applying a certain number of preparations on a piece of transparent glass and covered with glass objects, then observed. Preparations must demonstrate a homogeneous composition and no visible presence of grain [18,30].

Determination of pH

The pH determination was done by using a pH meter as follows: Tool first was calibrated using standard encompasses solution is neutral (pH 7.01) and the pH of the solution encompasses acidic (pH 4.01) to position the needle indicates the pH price above. The electrode was washed with distilled water, and dried with a paper tissue. A sample created in concentrations of 1% that weighs 1 gram of material diluted with distilled water to 100 ml in a container, and then the electrode was dipped in the solution, the needle left to move to a position of constant. The number shown in the pHmeter is pH price [18,30].

Determination of the power spread preparations

As many as 500 mg dosage was placed in the middle of the petri dish which has been fitted with a millimeter paper block. Then another covered with glass. Waiting for 1 minute and measured the diameter of the material of which spread from two sides [15,18].

Observation of the stability of the preparation

Each formula preparation was put into a transparent container closed part of it. Formula was stored at room temperature, and observed every week until 12 weeks. It was observed in the form of changes in consistency, color, smell, and the spread of material resources [18,30].

Testing irritation against the skin of volunteers

Irritation test performed on 6 people volunteer with how little material was applied on the back of ears volunteers, then left on for 24 hours and saw the changes that occur, if there was irritation of the skin will appear reddened, itchy, and rough. Criteria of volunteers who made the test panels on the irritation is as follows: 1. the able-bodied Women, 2. Age between 20-30 years, 3. Healthy physical and spiritual, 4. No history of allergy-related diseases, 5. Willing to volunteer [19,23].

Anti-aging Activity Test

Anti-aging activity test was done by using 12 volunteers and divided into four groups:

- a. Group I : 3 volunteers for blank formula
- b. Group II : 3 volunteers for gel formula with 1.0% grapefruit peel ethanolic extract
- c. Group III : 3 volunteers for gel formula with 1.5% grapefruit peel ethanolic extract
- d. Group IV : 3 volunteers for gel formula with 2.0% grapefruit peel ethanolic extract

All of test volunteers group measured the initial skin condition that includes: water contents (moisture), smoothness (evenness), pores, spots, wrinkles and the depth of wrinkles by using a skin analyzer. Then do the gel application until the gel spread on the back of the hand that has been marked, the gel is applied based on group that has been set. Gel application was done 2 times a day, every day for 4 weeks. Changes in skin condition measured weekly for 4 weeks by using a skin analyzer [24,28,37].

RESULTS AND DISCUSSION

The yield of grapefruit peel ethanolic extract was obtained 10% with a pH 5.6. Grapefruit peel ethanolic extracts obtained was blackish brown. This study used ethanol 96% as a solvent which can extract flavonoid compounds completely.

Water Content Results

The water content of the grapefruit peel simplicia at the level of 7.32% and meet the general requirement for simplicia that is not more than 10%. If the water content greater than 10% may be a fungal growth medium.

Phytochemicals Screening Results

Results of phytochemical screening of the grapefruit peel simplicia can be seen in (Table 2). Results of phytochemical screening known that grapefruit peel simplicia powder containing alkaloids are characterized with the formed of the precipitate after addition of reagents Mayer, Bouchardat and Dragendorff. Contains of saponins is characterized by the formation of foam after shaking and the foam does not disappear with the addition of HCl 2N. Contains of glycosides characterized by the formation of the purple rings after the addition of the reagent Molish and concentrated sulfuric acid. Contains of flavonoids characterized with the formation of intense red color of the border line of amyl alcohol. Contains of tannins characterized with the formation blue or black color after the addition of FeCl₃. Contains of steroids characterized by the formation of blue green color after the addition of reagents Lieberman Burchardat [25,40]. Simplicia have flavonoids as the secondary metabolites will have potential as antioxidants.

Table 2: Results of Phytochemical Screening Powder Simplicia

Screening	Reagent	Results (color / sediment)	Conclusion
Alkaloids	Dragendorff	(+) orange-brown	(+)
	Bouchardat	(+) yellow-kecoklatan	
	Mayer	(+) turbidity and a white precipitate	
Flavonoids	Zn + concentrated hydrochloric acid	(+) red	(+)
	Mg + concentrated hydrochloric acid		
Glikosida	Molish	(+) purple rings	(+)
	Fehling	(+) brick red precipitate	
	LB	(+) red	
Saponin	Hot water / shaken	(+) foam	(+)
Antrakuinon glikosida	NaOH	(+) intensively red on NaOH layer	(+)
Tanin	FeCl ₃ 1%	(+) green	(+)
Triterpenoid/Steroid	Liebermann-Burchard	(+) bluish green	(+) steroid

Description: (+) = containing the compound, (-) = Do not contain the compound

Antioxidant Activity Results

The maximum absorption of DPPH solution 40 ppm in ethanol was measured by using UV-Visible. The measured results showed that the DPPH solution in methanol to produce maximum absorption at a wavelength of 516 nm with

the absorbance at 516 nm wavelength 0.847, including in the wavelength range of visible light (400-750 nm). The wavelength range of DPPH solution about 515, 516, 517, 518, and 520 nm [14].

The antioxidant activity of grapefruit peel ethanolic extracts was obtained from measurements of the absorbance of DPPH in 60 minute with the addition of the test solution with a concentration of 40 ppm, 60 ppm, 80 ppm and 100 ppm compared with the controls DPPH (without the addition of test solution). In the results of the analysis of the grapefruit peel ethanolic extract (as the test sample) and vitamin C (as the comparison) antioxidant activity can be seen a decreasing in DPPH absorbance values to control at any increasing concentration. Absorbance was decreased and the DPPH trapping percent was increased with the increasing of the grapefruit peel ethanolic extract and vitamin C concentration. The results can be seen in (Table 3) and the regression equation and Inhibitory Concentration 50 (IC₅₀) shown in (Table 4).

Grapefruit peel ethanolic extract and vitamin C showed a decrease in the absorbance value at each increasing concentration. The decreasing in absorbance values was indicated greater antioxidant activity. Greater decreasing in absorbance values showed greater antioxidant activity. The decline occurred because the test solution traps the DPPH. The interaction of antioxidants with DPPH by transfer of electrons to the hydrogen atom of DPPH and will neutralize the DPPH free radicals. If all electrons in the DPPH free radical have been paired, it will be marked with the color changed of the solution from dark purple to yellow and absorbance at a maximum wavelength will be decreased [27,38]. Inhibitory concentration 50 (IC₅₀) values are obtained by calculating the linear regression equation obtained by plotting the concentration of the test solution and the DPPH antioxidant activity percent as parameters, wherein the concentration of the sample (ppm) as the X-axis and the value of % inhibition as the Y-axis. The results of the linear regression equation and inhibitory concentration 50 (IC₅₀) analysis results obtained from the grapefruit peel ethanol extract and vitamin C free radical trapping inhibitory concentration 50 (IC₅₀) value of 50% is used as a parameter to determine the antioxidant activity of the test sample [25,36].

Table 3: Absorbance and DPPH Trapping Percent by Grapefruit Peel Ethanolic Extract and Vitamin C

Test Solution	Conc. (ppm)	Absorbance			% Trapping			Average
		I	II	III	I	II	III	
Extract	40	0.512	0.509	0.516	39.551	39.906	39.079	39.512
	60	0.412	0.408	0.415	51.358	51.830	51.004	51.397
	80	0.289	0.330	0.323	65.880	61.039	61.865	62.928
	100	0.202	0.199	0.227	76.151	76.505	73.199	75.285
Vitamin C	2	0.772	0.774	0.769	8.855	8.619	9.209	8.894
	4	0.616	0.614	0.618	27.273	27.509	27.037	27.273
	6	0.459	0.456	0.458	45.809	46.163	45.927	45.966
	8	0.303	0.301	0.304	64.227	64.463	64.109	64.266

Table 4: The Regression Equation and Inhibitory Concentration 50 (IC₅₀) Analysis Results Obtained from Grapefruit Peel Ethanolic Extract and Vitamin C

Test Solution	Regression Equations	IC ₅₀ (ppm)
Grapefruit Peel Ethanolic Extract	$Y = 0.7426 X + 5.305$	60.182
Vitamin C	$Y = 18.4455 X - 0.571$	2.679

Gel Formulation Result

Homogeneity Preparations Result

The results of the experiments have been done on the preparation of the gel that is formulated using grapefruit peel ethanolic extracts with a variety of concentrations, there was no grain on a piece of glass, then the material is said to be homogeneous [10].

Determination of the pH Value Result

Preparations pH values determined using the pH meter that the average pH of the entire preparations tested ranged between 6,3–6,7 means eligible for material of skin moisturizer because the pH requirements for material of skin moisturizer is 5-8 [4,5].

Spread the Power Test Results

The results of measurements of the spread of power gel preparations formulated with different concentration of grapefruit peel ethanolic extracts between 5.10–5.28 cm means a good criteria for material gels because a good spread of power criteria for material of skin is 5-7 [15,18].

Stability Observation of the Preparation Result

Each gel preparation is obtained with the consistency of a thick gel preparation, with the addition of ethanol extracts of grapefruit skin respectively 1%, 1.5%, and 2% are brown to blackish brown, while the gel is colorless blanko (transparent). Gel preparations from all formulas are not going change in color, smell and shape after 12 weeks of storage at room temperature [15,18].

Irritation Test of the Volunteers

There was no the side effects in the form of redness, itching, and rough on the skin caused by the material. Then it can be inferred that the gel preparations formulated using grapefruit peel ethanolic extracts was not irritating to the skin [28,30].

Anti-aging Activity Test

Water Contents (Moisture)

The results of water content in the skin of all the groups can be seen in (Figure 1).

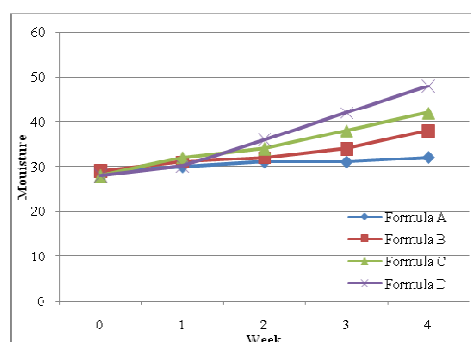


Fig. 1: Graph of moisture comparison of the skin of volunteers

From the test data shows that the water content of the skin, there was no increasing of water content of the skin after using of blank formula. Each formula with grapefruit peel ethanolic extract showed increasing of water content. In the group of volunteers who has been given grapefruit peel ethanol extract has increasing in the water content than a group of volunteers who has been given a gel formulation without extract (blank gel), in which the water content parameter measurement results if 0-29 (Dehydration), 30-45 (Normal) and 46-100 (Hydration). From the graph it can be concluded that the formula C further enhances water content percent than the formula B; and formula B further enhances water content percent than the formula A. But formula A, B and C are still in the criteria for normal skin.

UV rays are the biggest contributor to the formation of wrinkles. The onset of wrinkles caused decreased elasticity of the skin caused by the reduced water content of the skin and thickening of the stratum cornea [5,30]. Nutrition, activity and environment greatly was affected the water content in the epidermis and the dermis. The skin should be able to keep the moisture content to maintain its function as a healthy skin. If the moisture content decreased drastically, will cause skin to become dry, rough, and chipped [24]. Healthy skin has a distinctive: not easily absorb water, aqueous solution, or solid objects. Ability in absorbing skin (absorption) was strongly influenced by metabolism, moisture and the thickness of the skin [9,20].

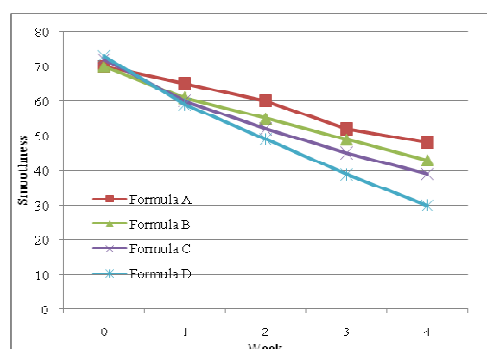


Fig. 2: Graph of smoothness comparison of the skin of volunteers

Smoothness (Evenness)

Evenness measurement results of all the groups of volunteers can be seen in (Figure 2).

Based on (Figure 2) above, it can be seen that all the groups of volunteers have normal skin to a prior administration of a preparation in which the evenness parameter measurement results if 0-31 results (smooth skin), 32-51 (normal skin), and 52-100 (rough skin). On the use of gel formulation of grapefruit peel ethanolic extract formula D indicates a change of skin becomes smoother after four weeks of application, the gel preparations with a formula A, B and C that showed no change in the skin condition for 4 weeks of usage.

Dry and coarse was also a common sign is experienced when the skin undergo premature aging. When the skin is too often exposed by sun rays, collagen and elastin in the skin layer will be the damaged, so the dead skin cells on the stack causes the skin surface korneum stratum being not smooth. As a result the skin was look more grainy. Skin feels rough, dull and flaky due to decrease in the ability of the skin to shed dead skin cells to be replaced with new skin cells [8,28].

Pores

Pore sizes measurement results of all the groups of volunteers can be seen in (Figure 3).

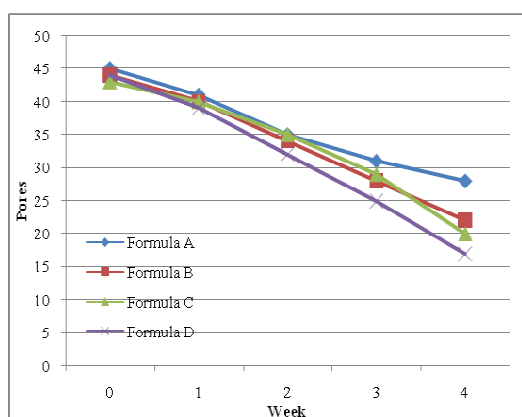


Fig. 3: Graph of pore sizes comparison of the skin of volunteers

In (Figure 3) it can be seen that the group of volunteers who used the gel of grapefruit peel ethanolic extract formula D have shown the pore size becomes small size after 4 weeks of application, other a group of volunteers who used the gel other formulas still shows the medium pore sizes, where the pores parameter measurement results if 0-19 pores (small), 20-39 (medium), and 40-100 (large). The group of volunteers who were given a gel formulation of all formulas had shown changes the pore size to medium after 4 weeks of usage.

One of the characteristics of a healthy face is the small pores. The pores will be enlarged when exposed to the rays of the sun shining. An enlarged pores causing dirt easy entry and clogged causing acne easier arising [28,41].

Spots

Number of spots measurement results of all the groups of volunteers can be seen in (Figure 4).

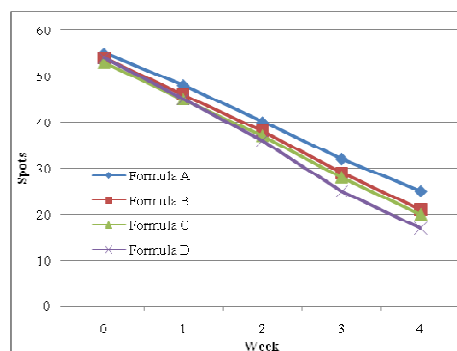


Fig. 4: Graph of spot numbers comparison of the skin of volunteers

From (Figure 4) shows that the usage of gel in each group gradually decrease the number of with the stain parameter measurement results if 0-19 (little), 20-39 (medium), 40-100 (lot). Decrease the amount of stain of the most well occur in a group of volunteers who used the gel peel extract of grapefruit formula D, at 4 weeks of usage decreased the number of spots becomes the little category, but the formula A, B and C after 4 weeks of usage still the medium category.

Black smudges (hyperpigmentation) usually appear on the skin begin to ripen as well as skin aging has not experienced by various causes. The cause of black flecks on the skin the most common is due to too much exposure to the Sun. The more frequently exposed to the Sun's rays on the skin, the more active the formation of melanin [29]. Melanosom contains brown bokroma is called melanin, as a determinant of skin color. Melanosom size is influenced by genetic factors and non genetic (exposure to sunlight). When the increased production of melanin will also increase and lead to spots and blemishes on the skin [28,42].

Wrinkles

Number of wrinkles measurement results of all the groups of volunteers can be seen in (Figure 5).

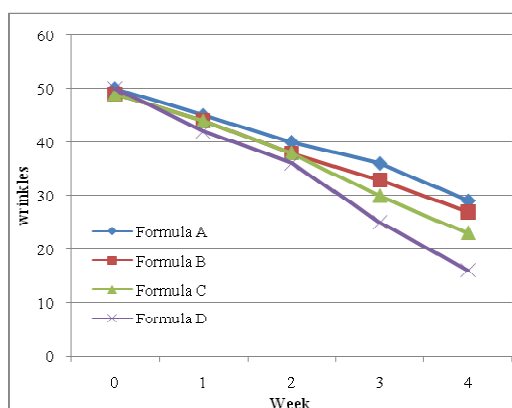


Fig. 5: Graph of number of wrinkles comparison of the skin of volunteers

(Figure 5) shows that all groups have wrinkled skin before the application where the wrinkles parameter measurement results if 0-19 (not wrinkled), 20-52 (wrinkled), and 53-100 (lots of wrinkled). During the 4 weeks usageshowed a decreasing in the number of wrinkles gradually after the use of the gel, but the formula A, B, and C have not been able to reduce the number of wrinkles to the not wrinkled skin condition, while the use of grapefruit peel ethanolic extract gel formula D in the fourth week had a reduction in wrinkles into the skin with not wrinkled criteria.

The process of aging was a natural process for everyone. The aging process that is highly visible to the naked eye is the occurrence of wrinkles on the skin (skin wrinkles). The occurrence of wrinkles on the skin prematurely and look old is another effect of ultraviolet rays. This effect could not be directed so wrinkles, but due to the accumulation of ultraviolet light in the long term that raises the effect of skin damage in fiber. When the damage to the fibers, the skin was begins to stretch and elastic, not losing its ability to return it into place after the stretch [9]. Wrinkles usually appear at some point in the area of the face, i.e., around the eyes, between the nose and mouth, cheeks, between the eyebrows and eyes, forehead, jaw line and neck [28].

CONCLUSION

Results of phytochemical screening in grapefruit peel powder simplicia gives a positive result for alkaloids, flavonoids, glycosides, tannins, saponins and steroids. The watercontents in the simplicia powder wasobtained 7.32% and eligible for extraction. The grapefruit peel ethanolic extract has a strong antioxidant activity category with IC_{50} values 60.182 ppm concentration. Gel formulation of grapefruit peel ethanolic extract at a concentration of 1.0%, 1.5% and 2.0% meet the requirements of homogeneity, good dispersive power from 5.10 to 5.28 cm, neutral pH from 6.3 to 6.7, stabilized in storage 12 weeks, and showed no skin irritation. Anti-aging activity test of the water content, evenness, pores, spots, and wrinkles, conducted over 4 weeks in which the grapefruit peel ethanolic extract with a concentration of 2% have the best anti-aging activity compared to other formulas. Blank formulations also performed and provide the results of the testing activities of anti-aging because it contains glycerin formulation form that serves as a moisturizer on the skin, but not as good as a gel containing grapefruit peeethanolic extracts.

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