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RESEARCH ARTICLE

Comparison of Ethanol Emulgel Extract Activity of Duku Fruit Peels (*Lansium domesticum Corr)* With Tranexamic Acid as a Skin Lightener

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Abstract

Objective: Emulgel which has the potential for skin lightener is a medical cosmetic preparation used to inhibit the formation of melanin pigments so that it can brighten the skin. The purpose of this study was to determine the effect of concentration variations of duku (*Lansium domesticum* Corr.) peel waste extracts on the evaluation results of preparations, the level of irritation to rabbits, and an increase in brightness on human skin compared with emulgel containing tranexamic acid. A dermatoscopic test is used to look at the imaging of the skin surface after the use of an emulgel. Material and Method: In this study, concentration variations of ethanol extract from duku peel waste of 10%, 15% and 20% were used. Result: The test results obtained that all formulas meet the requirements of organoleptic test, pH, homogeneity, dispersibility, and centrifugation test. Irritation test with the *Draize* method obtained all formulas do not irritate. The results of testing the increase in skin brightness on emulgel preparations showed that F1 (16.67%), F2 (33.33%), F3 (60%), F4 (60%). Conclusion: Increasing the brightness of emulgel preparations containing 20% ethanol extract of duku peel is comparable to 4% tranexamic acid.

Keywords: *Tyrosinase, Lightening cream, Duku fruit Peel, Tranexamic acid.*

Introduction

Melanin is the main pigment responsible for the pigmentation of human skin, hair and eyes, produced by melanocytes through melanogenesis. Melanin has a photoprotective function in human skin, but overproduction of melanin can cause aesthetic problems and serious illnesses related to hyperpigmentation. Inhibition of the formation of melanin can result in a reduction in skin darkness. The formation of melanin in the human body can be reduced by several mechanisms, including the use of antioxidants, direct tyrosinase inhibition, inhibition of melanin migration from cell to cell and hormonal activity [1].

Tyrosinase is a copper-containing enzyme that catalyzes two rate limiting reactions in melanogenesis, namely hydroxylation of monophenol to o-diphenol, and oxidation of o-diphenol to o-quinone. Therefore, tyrosinase inhibition is the main target for researchers to regulate melanin production. Many tyrosinase inhibitors have been used as skin lightening agents but most of them have weaknesses. Hydroquinone causes effects such as irritation and burning on the skin, mutagenic cells in mammals and cytotoxic in melanocytes. Kojic acid and arbutin also show poor efficacy *in vivo*, unstable and low skin penetration, and the use of kojic acid in skin care is very limited due to its carcinogenicity. Ascorbic acid tends to degrade and ellagic acid has very poor bioavailability. Thus the development of better tyrosinase inhibitors is needed [2]. Flavonoids are natural polyphenols that are found in leaves, stems and flowers.

The ability of skin depigmentation from flavonoids by directly inhibiting tyrosinase activity in the melanogenesis process. Flavonoid bonding with copper (Cu) which is an active site of the tyrosinase enzyme due to the presence of hydroxyl groups in rings A and B in flavonoids (OH groups in C6-C8 and

C4) and their antioxidant effects are reported to play a role in inhibiting the action of the tyrosinase enzyme [3, 4].Duku plants (*Lansium domesticum* Corr) are seasonal plants that grow in tropical regions, especially Indonesia. During the fruiting season, the peel of the duku fruit only becomes waste in the environment, whereas its utilization can be increased if the potential for bioactivity is explored. Based on research that total flavonoid content in ethanol extract of duku fruit peel was 4.76 ± 0.2% w/w equivalent to quercetin and antioxidant potential had IC_{50} value of 6.53 mg /L by DPPH method [5].

Clinical trials of ethanol extract of duku fruit using mexameter has been shown to reduce the skin melanin index by 1.8 units after twice daily use for 4 weeks [6]. In addition, tranexamic acid is a relatively new developed substance in the treatment of hyperpigmentation (melasma). Intracellular release of arachidonic acid (AA) which is a prostanoid precursors and increased alpha-melanocyte-stimulating hormone is a result of plasmin activity, both of which can activate melanin synthesis.

Therefore the antiplasmin activity of tranexamic acid is considered as the main mechanism on the hypopigmentation effect [7]. Based on research by Kanechorn [8], it was proven that 78.2% of melasma patients showed a decrease in the melanin index on their face after being given 5% topical tranexamic acid twice daily for 12 weeks.

Other studies have shown the use of 5% topical tranexamic acid to be very effective and safe, with good patient acceptance for the treatment of melasma which has fewer side effects such as erythema, irritation, and telangiectasia compared to the use of three combinations of hydroquinone, tretinoin, fluocinolone [9].Based on these data, In this research, an emulgel preparation was made containing ethanol extract from Duku peel waste and tranexamic acid as comparison. Furthermore, preparations carried out by evaluation, safety tests and testing for their effectiveness as a skin lightener.

Materials and Methods

Maserator, rotary vaccum evaporator (Eyela®), water bath, Moisture Balance, pH meter, Viscometer (Brookfield®), Centrifuge (OneMed Health), Skin Color Chart, Video Dermatoscope (Dino-Lite), Duku (*Lansium domesticum* Corr), tranexamic acid (Merck)), carbomer (Merck), aqua deion, Tween 80 (BASF), TEA (Brataco), glycerin (Brataco), DMDM Hydantoin (Merck), Plantacare (Merck), ethanol 70% (Brataco), Sodium lauryl sulfate (Brataco).

Sample Preparation

Determination of plants carried out at the Herbarium Laboratory at Padjadjaran University which aims to prove the duku fruit obtained is really a plant that is in accordance with the literature. Duku fruit is obtained from the Singaparna market-Tasikmalaya. Duku fruit taken and sorted is fresh yellow skin. Then the skin of the Duku fruit is washed and dried under indirect sunlight until the water content <10%. Simplicia of Duku fruit peel was milled into powder using blender and then sieved with a mesh size 60.

Duku fruit peel powder was extracted by maceration method using 70% ethanol solvent for 3 x 24 hours. The maceration results are filtered; the solvent is evaporated with a rotary evaporator at 60°C, then dried over a water bath until it becomes a thick extract.

Simplicia and Extract Examination

Simplicia examination includes water content and total ash content test. Phytochemical simplicia screening and extracts include alkaloid, flavonoid, saponin, tannin, and triterpenoid tests.

Formulation of Emulgel Ethanol Extract Duku Fruit Peel and Tranexamic Acid

In this study, the formulation of emulgel preparations used 3 variations in the concentration of ethanol extract of duku peels, namely 10% (F1), 15% (F2) and 20% (F3) and tranexamic acid with a concentration of 4%. The preparation formula can be seen in Table 1.

Table 1: Composition of Emulgel Ethanol Extract Duku Fruit Peel and Tranexamic Acid

Name of substance	Formula (% w/v)			
	F1	F2	F3	F4
Ethanol extract of duku peel	10	15	20	-
Tranexamic Acid	-	-	-	4

Tween 80	15	15	15	15
Plantacare	10	10	10	10
Carbomer	7	7	7	7
TEA	qs	qs	qs	qs
Glycerin	15	15	15	15
DMDM Hydantoin	0.5	0.5	0.5	0.5
Sodium metabisulfite	0.01	0.01	0.01	0.01
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

Evaluation of Skin Lightening Emulgel

- Organoleptic

Examinations include visually observed color, odors, and dosage forms [10].

- Homogeneity Test

Homogeneity checks are carried out using a glass object by means of a certain number of preparations smeared on a piece of glass and viewed under a 100x magnification microscope, resulting in homogeneous preparations and no visible granules [10].

- Determination of pH

The preparation is weighed as much as 1 g and dissolved in 100 mL distilled water. Then the electrode is dipped in the solution, until the instrument shows a constant pH value. The number indicated by the pH meter is the pH of the preparation [10].

- Viscosity Test

This viscosity test aims to determine the level of thickness of the cream preparation. In this viscosity measurement the Brookfield Viscometer is used by using spindle no. 7 at 100 rpm [11].

- Scattering Test

0.5 gram of emulgel from the formulation was weighed and placed on a slide that had been coated with graph paper and on top of it was given another slide, left for 1 minute. Furthermore, given a load of 50, 100 and 150 grams respectively, left for 60 seconds. The diameter of the resulting distribution is measured using a ruler [10].

- Centrifugation Test

A total of 10 grams of emulgel was put into a centrifugation tube and centrifuged at 3750 rpm for 5 hours. After centrifugation, it is observed whether or not separation occurs [12].

Irritation Test for Skin Lightening Emulgel

The irritation test of emulgel preparations was carried out on 5 rabbit test animals using the Draize method. On the back of the rabbits, 6 patterns are made using markers, which are 3 sections each on the right and left in the shape of a rectangle with a size of 3 x 2 cm with a distance between the parts of 2 cm. Feathers are shaved smooth on a pattern that has been made, then cleaned and smeared with 95% ethanol. In the pattern the SLS 15% solution was treated as positive control, the basis of the emulgel (F0) formulation was negative control, and formulas (F1, F2, F3, and F4) as samples.

Each 0.5 g sample is applied to the back of the rabbits that have been shaved, then covered with sterile gauze and then tied with a long bandage around the stomach and back for 24 hours. After 24 hours, the bandages and bandages are removed and left for 1 hour, and observed. Then the part is covered again with the same plaster and the observations are carried out again after 48 hours. The assessment for each skin condition is given a value as shown in Table 2. And the irritation index is calculated by adding up the values of rabbits after 24 hours and 72 hours of irritant sampling, then dividing the 4 irritation assessments which can be seen in Table 3 [13].

Table 2: Evaluation of Irritation Tests

Erythema levels	value	Edema Levels	value
	0		
No erythema	1	No edema	0
Very Slight Erythema	2	Very Slight edema	1
Well-defined erythema	3	Well-defined edema	2

Moderate-to-severe erythema	4	Moderate-to-severe edema	3
Severe erythema		Severe edema	4

Table 3: Irritation index

Irritation Level	Result
Practically non-irritant	0.00
Slight-Irritant	0.04-0.99
Mild-irritant	1.00-2.99
Moderate-irritant	3.00-5.99
Severe-irritant	6.00-8.00

Test the Effectiveness of Skin Lightening Emulgel Preparations on the Skin

This test is carried out by 5 probandus in each formula. Criteria as a probandus include able-bodied women; aged between 18-30 years, there is no history of diseases associated with allergies to the skin and is willing to become a probandus by filling out the readiness form as probandus. This emulgel preparation test is done by applying preparations on the back of the left hand with an area of 5x5 cm every morning and night for 2 hours for 7 days and observed once every day. Observations of the results is done by directly observing physical changes including changes in the level of skin color brightness and match it with the color number on the skin color chart. In this test the back of the right hand was used as a control [14].

Skin Imaging Surface Test Using Dermatoscope Videos

Skin surface imaging tests are performed using Dermatoscope Videos. The test was conducted on 6 female volunteers aged over 40 years. Volunteers will use preparations on certain parts of the skin every day for four weeks and evaluation is done every week. The results of surface imaging of the skin can be seen by measuring the width of the volunteer skin wrinkle lines [15].

Results and Discussion

Simplica duku fruit peel in this study contains a moisture content of 6% and a total ash content of 1.4%.

So that it can be said that simplicia meets the requirements of water content must be less than 10% and the total ash content is not more than 2% [16]. The total ash content test is used to determine the overall amount of minerals contained in the simplicia of the skin of the duku fruit (*Lansium Domesticum* corr.) According to Sudarmadji et al., ash is a mixture of inorganic components or minerals found in a food or agricultural waste from the combustion of an organic material [17].

Simplicia was milled using blender until it becomes powder and sieved with a 60 mesh sieve. The results of the simplicia powder of duku fruit peel obtained of 768 grams were then extracted by maceration method using 70% of ethanol solvent. The maceration method was chosen because it avoids damaging the compound due to the heating process.

Maceration method is done by immersing simplicia with organic solvents (70% of ethanol) for 3 x 24 hours, through immersion cell breakdown will occur due to differences in pressure between inside and outside the cell so that secondary metabolites present in the cytoplasm will be dissolved in organic solvents. From the extraction process, the yield of duku peel extract was 9.1%. Phytochemical screening results show that simplicia and ethanol extract of duku fruit peels contain alkaloid, flavonoid, saponin, and triterpenoid compounds. The results of phytochemical screening of simplicia and extract can be seen in Table 4.

Table 4: Simplicia Phytochemical Screening and Extracts

Test Compounds	Simplicia	Extracts
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Tannin	-	-
Triterpenoid	+	+

Notes: (+) Present, (-) Absent

Ethanol extract of duku fruit peel and tranexamic acid was formulated in the form of emulgel preparation, by making oil/water (O/W) emulsion then mixed with gelling agent. The superiority of emulgel is that it provides comfortable use and excess good conductivity of drugs such as gels and emulsions.

Evaluation of skin lightening emulgel preparations was carried out to see stability during storage, including organoleptic observations show that the emulgel preparations has semisolid form, and did not show changes or phase separation during the 28-day storage period. Organoleptic evaluation results can be seen in Table 5. Homogeneity test is done to find out that the preparations are made homogeneous and free of particles that are still clumping [18]. Homogeneity testing is done by putting all preparations of emulgel formula between a piece of glass and transparent material. The results show that all formulas are homogeneous and there are no coarse particles visually. The homogeneous final product is affected by stirring during the manufacturing process which can increase the speed of homogenization. Then centrifugation test is performed, the results show there is no phase separation in all formulas.

The pH measurement of the emulgel preparation was carried out at room temperature in order to find out whether the emulgel preparation in this study was in accordance with the pH of the skin which is 4.5 - 6.5 [19]. Measurements were made using a pH meter with a period of 28 days. Based on the test results there was a decrease in the price of pH in formulas 1, 2 and 3 compared to formula 4. The results of checking the pH of the preparations can be seen in Table 5.

Table 5: **Results of pH determination**

Preparations	pH of day preparation				
	0	7	14	21	28
F1	5.7	5.6	5.4	5.4	5.2
F2	6.1	6.0	5.7	5.6	5.6
F3	5.1	5.1	5.0	5.0	4.9
F4	5.4	5.4	5.4	5.4	5.4

Viscosity test aims to determine the level of viscosity of the emulgel preparation. Testing is carried out for 28 days. Viscosity of preparations F0, F1, F2, F3 and F4 was tested using Brookfield Viscometer with spindle no. 7 at a speed of 100 rpm produced good viscosity of preparations. Viscosity test results can be seen in Table 6.

Table 6: Viscosity test results

Preparations	viscosity of stock (cps) Day-to				
	0	7	14	21	28
F1	16400	16480	16490	16500	16500
F2	15920	15910	15880	15880	15880
F3	18960	18940	18850	18830	18830
F4	18880	18670	18490	18490	18330

The spread test was carried out to determine the extent of spread of skin lightening preparations on the skin. The scatter power test is carried out using a load of 50 grams, 100 grams, 150 grams in successive tests. Dispersal surface that is produced by increasing the load can describe a characteristic on the emulgel.

Table 7: Spread Power Test Results

Preparations	Load (grams)		
	50	100	150
F1	2.5 cm	3.2 cm	3.5 cm
F2	3.5 cm	4.7 cm	4.9 cm
F3	2.7 cm	3.8 cm	4.4 cm
F4	2.1 cm	2.4 cm	3.0 cm

The spread ability of semisolid preparations can be divided into 2, namely semi stiff (high viscosity) with a range of 3-5cm, and semifluid (low viscosity) with a range of 5-7cm [10].Table 7 show that all formulas are semistiff in the range 3-5cm. The greater the

viscosity value, the smaller the spread power of a preparation. Centrifugation test is carried out using a centrifugator, aiming to observe the separation or not visually [12]. Emulgel preparations were centrifuged at 3000 rpm for 30 minutes showing the results that for all formulas there was no separation. In this skin lightening preparations, there are surfactants namely tween 80 which can increase solubility so as to avoid the separation of the preparation phase during storage.

This irritation test was carried out on as many as 5 rabbit test animals, observations were carried out at the 24th hour and 48th hour. In the positive control which is a sodium lauryl sulfate (SLS) solution with a concentration of 15%, the primary irritation index value obtained was 5, 10 (moderate irritation). Continuous rabbit skin exposure to SLS can cause mild to moderate irritation.

While the negative control (F0), F1, F2, F3 and F4 produce an irritation index value of 0, based on the category of response the irritation index is not irritating (0.00). The preparation test for skin lightening was carried out on the back area of the human hand for 14 days. The test results show that all formulas (F1, F2, F3 and F4) have increased brightness, marked by the different skin color of the left hand back used as a test, as well as when compared with the Skin Color Chart on each formula.

Discoloration of the skin on the back of a volunteer's hand is a process of inhibiting melanin synthesis. The results of skin brightness testing using the skin color chart can be seen in Table 8. Melanin is a pigment in the skin that is synthesized by melanosome, there are 2 types of melanin namely eumelanin gives a dark color (black-brown) and pheomelanin gives a bright color (red-yellow). Melanogenesis is the process of forming melanin in cells.

The main enzymes involved in the melanogenesis pathway are tyrosinase, tyrosinase related protein 1 (Tyrp1), known as gp75 glycoprotein, and tyrosinase related protein 2 (Tyrp2), also known as dopachrome tautomerase. The melanogenesis process is started by hydroxylation of phenylalanine to L-tyrosine. L-tyrosine is hydroxylated to L-dihydroxyphenylalanine (L-DOPA) and then L-DOPA is oxidized to L-DOPAquinone (DQ), in which both processes are catalyzed by the enzyme tyrosinase. If cysteinyl or glutathione is present, it reacts with DQ to become cysteinyl DOPA and will be oxidized and also polymerized into feomelanin. If there are no thiol compounds (cysteine and glutathione or thioredoxin), DQ will change to DOPAchrome. Tyrp 2 catalyzes the tautomerization of DOPAchrome to 5, 6-dihydroxyindole-2-carboxylic acid (DHICA or brown eumelanin), which is then oxidized into DHICA-melanin subunits. The oxidation of DHICA to eumelanin is thought to be catalyzed by Tyrp 1. In the absence of Tyrp-2 the carboxylic acid group from DOPAchrome spontaneously disappears to form 5,6-dihydroxyindole (DHI or black eumelanin).

DHICA and DHI are eumelanin subunits. The level of brown to black eumelanin is the DHI / DHICA ratio, high ratio will tend to form black, while low levels will form brown [20]. In addition, ultraviolet radiation increases the production of reactive oxygen species (ROS) in keratinocytes and melanocytes, and at high concentrations ROS causes DNA damage and activates p53 resulting in increased expression of POMC (pro-opiomelanocortin), which is then divided into small peptides, such as ACTH, α-, ß-, and γ-MSH. α-MSH derived from POMC stimulates melanocortin-1 receptor (MC1R) in melanocytes further stimulates the formation of cyclic adeno monophosphate (cAMP) which activates tyrosinase and then triggers melanocyte proliferation and ultimately increases melanin production [21].

Emulgel of extract of duku fruit peels and tranexamic acid emulgel can function as tyrosinase inhibitors in the formation of eumelanin. Tyrosinase inhibitors bind covalently to the tyrosinase enzyme which causes the tyrosinase enzyme to not bind to its substrate (L-tyrosin) so that there is no excessive formation of melanin. If the formation of melanin is not excessive then there will be no pigmentation or skin damage [22].

The process of the mechanism of inhibition of the enzyme tyrosinase by duku peels extract comes from secondary metabolites contained, namely flavonoids. The flavonoid compound is one of a group of polyphenol compounds, where in the polyphenol compound there is a phenol ring that binds to the hydroxyl group.

The greater the number of phenolic hydroxyl groups, the better the antioxidant activity. Phenolic hydroxyl groups can inhibit the activity of the tyrosinase enzyme and inhibit lipid peroxidation, in which the presences of two hydroxyl groups located in the aromatic rings at positions 2 and 4 in the flavonoids play a role in the inhibition of tyrosinacetroase activity. So that the structure of flavonoids is compatible with the role of substrates and inhibitors [22, 23].

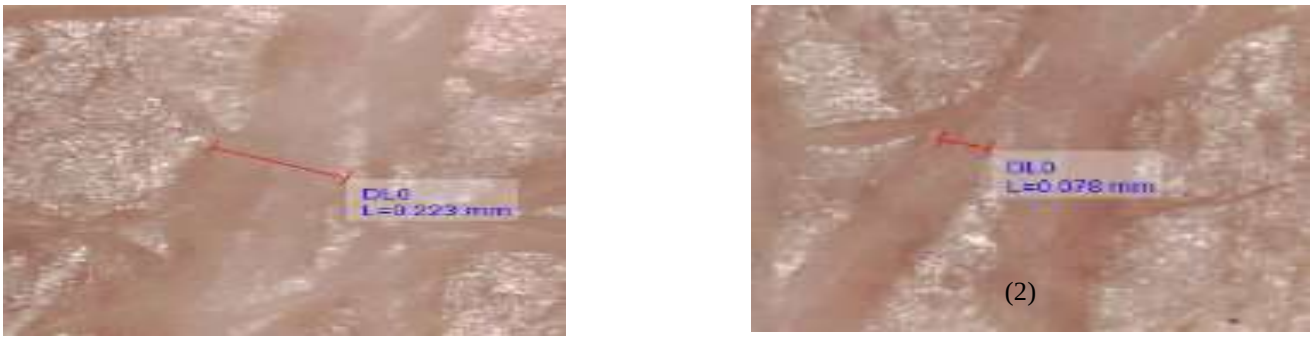
While tranexamic acid can inhibit the process of melanogenesis through the plasminogen activator pathway to plasmin, melanocytes, and keratinocytes. An increase in plasmin can increase α-melanocyitestimulating hormone (α-MSH), which activates melanin synthesis in melanocytes through transcription of the melanogenic tyrosinase enzyme.Tranexamic acid does not affect the number of melanocytes, but on the release of melanin [24].

Table 8: Results of Skin Brightness Testing with Skin Color Chart

Probandus	Information	Days to-					Percentage Increase in Brightness (%)
		1	3	5	7	14	
1	F1	6	6	6	5	5	16.67
2	F2	6	6	5	5	4	33.33
3	F3	5	5	4	3	2	60
4	F4	5	5	4	3	2	60

Figure 1: *Skin Colour Chart* [14]

Furthermore, for formula 3 which shows the result of increasing brightness which is proportional to 4% tranexamic acid, skin surface imaging test is performed using a video dermatoscope. The results showed that there were differences in texture of wrinkles on the skin on first and fourth day. On fourteenth day the imaging test showed that the width of volunteer skin wrinkle lines was reduced when compared to first day. So that after using F3 for 14 days not only increases the brightness of the skin but can also improve the texture of the skin wrinkles becoming smoother. The results of skin imaging testing with a dermatoscope video can be seen in Figure 2.



(1)

Figure 2: Test Results for Skin Imaging Testing with Video Dermatoscope (1) Day 1 (width 0,233 mm); (2) Day 14 (0.078 mm width)

Conclusion

The test results obtained that all formulas meet the requirements of organoleptic test, pH, homogeneity, dispersibility, and centrifugation test. Irritation test with the Draize method obtained all formulas do not irritate. The results of testing the emulgel preparations for an increase in skin brightness showed that F1 (16.67 %), F2 (33.33 %), F3 (60 %), F4 (60 %). Increased brightness of the skin from emulgel preparations containing 20% ethanol extract of duku fruit peels is proportional to 4% tranexamic acid.

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