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ANTIOXIDANT CAPACITY TEST & QUALITY EVALUATION OF HAND BODY LOTION *KEDONDONG* JUICE (*Spondias dulcis Parkinson*)

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ABSTRACT

Kedondong (Spondias dulcis Parkinson) is a tropical fruit known for its rich vitamin C content and associated antioxidant activity. The challenges will maximize the benefits of vitamin C in kedondong fruit soluble in water, and the light will degrade and reduce its effectiveness as an antioxidant. Previous research has highlighted that the heating process affected the antioxidant activity and the ethanol extract kedondong is used to make hand body lotion. This research aims to exploit the potential of using kedondong fruit in the formulation of hand body lotion by utilizing its antioxidant activity. The study used the FRAP method using Trolox as a synthetic antioxidant so that the antioxidant capacity of kedondong juice hand body lotion is obtained which was equivalent to TEAC (Trolox Equivalent Antioxidant Capacity). The results obtained from the hand body lotion preparation of kedondong fruit juice were 4.5 mM TE/gram, while kedondong fruit juice was 23.04 mM TE/gram. The results of the quality evaluation test of hand body lotion preparations have good homogeneity, do not cause irritation, have stable pH, and have a moisturizing effect on the skin.

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INTRODUCTION

Indonesia is a country rich in natural resources and has a variety of nutritious plants. Kedondong from the Anacardiaceae family is a tropical plant that has leaves, bark and fruit can be used for medicine (Yanti et al., 2023). Kedondong fruit contains vitamins A, B, C, carbohydrates, fat and protein. Vitamin C is a substance that is useful as antioxidant (Sudewi et al., 2021). Antioxidants protects the skin from damage, can suppress free radicals, which damage barrier skin and human body cells from a variety of disorders (Nst, M.R., dkk. 2023). Research by Sugiyanto dkk (2022) found drying kedondong simplicia at various temperatures affected antioxidant activity and methanol extract was measured by the DPPH method. Sudewi et al. (2021) made a hand body lotion from ethanol extract as a skin moisturizer and produced hand body lotion with the best formula at a concentration of 3.5%. This research has not tested its antioxidant activity, so that the hand body lotion from juice kedondong fruit is one of the good alternative natural ingredients to prevent damage and moisture skin cells and antioxidants activity is measured by the FRAP method. Antioxidants, which protect the skin from damage, can suppress free radicals, which damage human body cells from a variety of disorders. In this study, a test was conducted on the ability of kedondong flesh and hand body lotion in protecting the oxidation reaction to the lipids and metal minerals required by the body (Rumpf et al., 2023).

Methods

Tools and Materials

Tools

Digital scale (Kern ABS 220-4N), blenders (Philips), pH meters (Metrohm 744), skin analyzer checker (CR-302), UV-Vis spectroscopic photometry (T70), centrifuges (Hettich EBA 200), vortex (*Heidolph REAX control*), measuring pipettes (Iwaki), volume pipets (Iwaki), measuring glasses (Pyrex), spray cloves, tube shelves, suction balls (D&N) and labor glass tools.

Materials

Kedondong purchased from Padang city, West Sumatra Province, aquades, 96% ethanol, stearic acid, nipagin, nipasol, Stearyl alcohol, Trietanolamine (TEA), perfume, Troloks (Aldrich), HCl, TPTZ (Sigma), Sodium acetate (Emsure), NaOH, FeCl3 (Emsure).

1. Sample identification

Identification of samples was carried out at the ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Padang.

2. Juice preparation

Kedondong were peeled from the skin, separated between the flesh and the seed. Then it is thoroughly washed using an aqueduct, drained and sliced. Weigh as much as 100 g of the

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flesh, cut small and add 200 mL of aquades (1:2) and mashed using a blender. After that, strain using a clean flannel to separate the juice from the pulp. Then a centrifuge is used to get clear juice. The solution is stored in a dark, airtight container and stored at cold and dry temperatures (Shah et al., 2009)

3. Formula hand body lotion from *kedondong* juice (Sudewi et al., 2021)

Tabel 1. Composition of hand body lotion

Ingridient	Composition (g)
Kedondong juice	3,5
Stearic Acid	3
Stearylalcohol	1
Nipagin	0,1
Nipasol	0,15
TEA	0,75
Perfume	q.s
Aquadestill	100

4. Making *hand body lotion*:

a. Put stearic acid, cetyl alcohol in a dish of vaporizer, then melted on a water bath at 70°C until all melted (mass 1)
b. TEA mix with water at about 70°C in beaker glass (mass 2)

On other glass beaker:

1. Dissolve nipagin using some hot water.
2. Dissolve nipasol using some hot water.
3. Then mix the two preservative solutions (mass 3)

c. In a hot mortar, enter mass 1, mass 2 and mass 3 while grinding constantly until homogeneous and the temperature adjusts the room temperature, then a *hand body lotion base is obtained*.
d. Then added *kedondong*juice with a predetermined concentration so that *hand body lotion* is obtained.

5. Preparation of Antioxidant Activity Test Reagents

a. Preparation of Acetate buffer solution pH 3.6.
   Weigh 1,550 g of sodium acetate trihydrate on the watch glass. Dissolve in 8 mL glacial acetic acid in a 500 mL measuring flask, then suffice using an aqueous to the limit mark. The solution is stirred and its pH is measured. The desired pH setting is 3.6 using NaOH or HCl.
b. Preparation of $FeCl_3.6H_2O$ solution 20 mmol/L

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Weigh 0.2703 g of $FeCl_3.6H_2O$ powder on the watch glass. Dissolve using aqueous in a 50 mL measuring flask. Then, stir and transfer into a dark reagent bottle and mark with a label containing the substance name, concentration and date of manufacture.

c. Preparation of 40mM HCl solution
   Pipette 0.17 mL of 37% HCl solution and put it in a 50 mL measuring flask that contains a small amount of aquades, then add aqueous to the limit mark. The solution is stirred and transferred into bottles of dark reagents and marked with a label containing the substance name, concentration and date of manufacture.
d. Preparation of TPTZ (2,4,6-tripyridil-striazine) solution 10 mmol/mL
   Weigh 0.031 g of TPTZ powder on the watch glass. Dissolve the powder using 40mM HCl in a 10 mL measuring flask. The ocean is stirred and transferred into a dark reagent bottle and marked with a label containing the substance name, concentration and date of manufacture.
e. FRAP reagent setup
   Made a mixture between acetate buffer: $FeCl_3.6H_2O$: TPTZ with a comparison of 10 : 1 : 1 in beaker glass.
f. Preparation of Trolox 1000 µMstandard's solution.
   Dissolve 25,029mg of Trolox using 96% ethanol in a 100 mL measuring flask and then dilute to the limit mark and store in the refrigerator.

6. Qualitative Analysis of Antioxidant Activity Using FRAP Method (Haryoto & Frista, 2019)

Pipet the sample solution of 1 mL using a volumetric pipette, insert it into the test tube and add 3 mL of FRAP reagent. The mixture is homogenized and incubated at room temperature for 30 minutes. Observe the color changes that occur against the mixture. When it turns blue, it can be said that the sample has activity as an antioxidant.

7. Quantitative Analysis of Antioxidant Activity Using FRAP Method

a. Determination of the maximum absorption wavelength of Trolox + FRAP
   The maximum wavelength is obtained by measuring the absorbance of the Trolox standard solution (300 µM). The solution is pipetted as much as 1 mL, then read at the maximum absorption wavelength using a UV-Vis spectrophotometer.
b. Preparation of standard solutions Trolox 200, 300, 400, 500, 600 µM
   The mother liquor of Trolox is pipetted 1 each; 1,5; 2; 2,5; and 3 mL. Put in a 5 mL measuring flask and add 96% ethanol to the limit mark so that the concentration is obtained.
c. Trolox calibration curve manufacturing
   Each Trolox concentration was pipettes of 1 mL and 3 mL FRAP reagents to the test tube. Then the mixture is homogenized and incubated at room temperature for 30 minutes and the absorption at the maximum absorption wavelength.
d. Antioxidant analysis of *kedondong* juice

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Kedondong juice sample solution (1: 2) pipette 0.5 mL, diluted into a 10 mL measuring flask. The sample solution was pipetted 1 mL and 3 mL of FRAP reagent, was incubated at room temperature for 30 minutes and measured absorbance. If the absorbance does not meet the requirements, it is diluted using aquades in a 10 mL measuring flask until the absorbance is in the rangeof 0.2 – 0.8.

e. Antioxidant analysis of hand body lotion
Weighing as much as 0.5 g of hand body lotiondissolve using aquades on a 10 mL measuring flask. Take 1 mL of the solution, add 3 mL of FRAP reagent, and measure the absorbance in the range of 0.2 – 0.8.

8. Data Analysis

Antioxidant activity is calculated using values TEAC (Trolox Equivalent Antioxidant Capacity).Based on absorbance data and equations y = a + bx and used formula:

$$TEAC=\frac{Vsample(mL)\times[sample]\times fp}{weight(gram)}$$

Note
TEAC : Trolox Equivalent Antioxidant Capacity
[sample] : Sample concentration
fp : Diluting Factor

9. Evaluation Quality of hand body lotion

a. Homogeneity test
The preparation of hand body lotion is applied to the glass object, then observed coarse particles under a microscope. If coarse particles are found, the sample is declared inhomogeneous, while if no coarse particles are found, the sample is declared homogeneous(Sudewi et al., 2021).

b. pH Test
The preparation of hand body lotionis measured pH using a pH meter by means ofweighed as much as 1g, then diluted into a 100mL measuring flask. The preparation should not exceed the pH of the skin, which is 4.5 – 8 (Irmayanti et al., 2021).

c. Irritation test
The preparation is applied thinly to parts of the body that are rarely touched such as on the skin behind the ears or elbow folds of the hands. Then observed symptoms such as itching and redness (Sudewi et al., 2021).

d. Test moisture effectiveness
The preparation is used by several volunteers for 4 weeks. The results were measured using a skin analyzer checkertool every week.

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RESULTS AND DISCUSSION

Based on the composition of Indonesian food plants, from 100 grams of fresh kedondong flesh, there is 58% edible fruit flesh and contains Vitamin C of 32 mg with a water content of 87.8% (Kemenkes R.I.2020). The content of vitamin C in the form of a solution is easily oxidized in the presence of air (Shah et al., 2009). The high water content in the flesh of the kedondong fruit makes it difficult to dry the flesh kedondong which tends to change the colour of the slices of fruit flesh from day to day, so based on this, the withdrawal of fresh juice is carried out and immediately measured antioxidant activity with the FRAP (*Ferric Reducing Antioxidant Power)* method. The selection of this method is a simple, easy general method that does not require special tools but can detect an antioxidant (Yuliawati, 2022), (Wabula et al., 2019) Antioxidants from kedondong juice will reduce Fe^{3+} compounds to Fe^{2+} which can be seen by changing the colour of the solution (figures 1 and 2) (Haryoto & Frista, 2019). In this study, the synthetic antioxidant Trolox was used to see the ability of Ferric ions in FRAP reagents to be reduced by Trolox to blue Ferro ions. Then it will be done on kedondong juice which also has antioxidant power. The use of Trolox as a synthetic antioxidant in this study is due to it being one of the antioxidant synthesis compounds derived from water-soluble α-tocopherol. The ability of Trolox as an antioxidant to inhibit the peroxidation of unsaturated fatty acids, the formation of radical oxygen atoms, and superoxide anion in cells can prevent cell damage (Zubov et al., 2023).

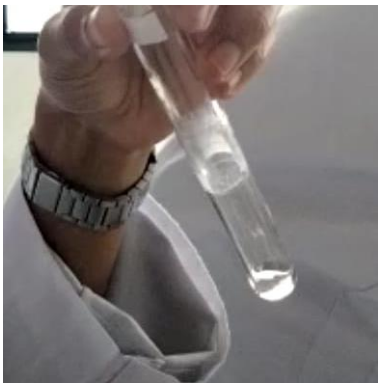

Figure 1. Sample solution color before FRAP is added

Figure 2. Sample solution color after FRAP is added

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To obtain the maximum absorption wavelength, Trolox and FRAP are measured at a concentration of 300 µM trolox mixed with FRAP 1:3. The mixture is homogenized and incubated at room temperature for 30 minutes. Maximum wavelength measurement results at 594 nm.

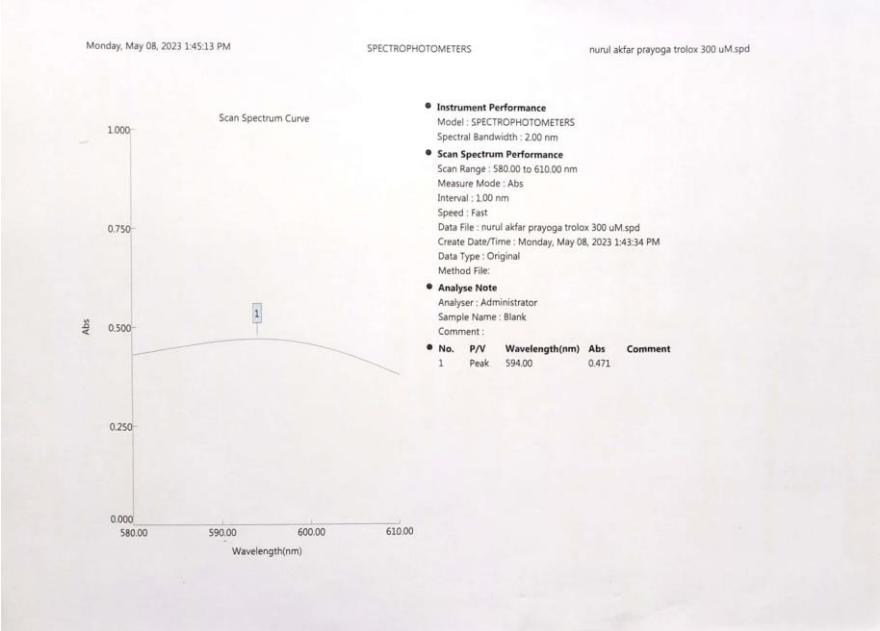

Figure 3. Trolox and FRAP Maximum Absorption Wavelength Curve

The Trolox calibration curve equation is obtained from a number of Trolox solution concentrations, are 200, 300, 400, 500 and 600 µM. Every 1 mL of each concentration of Trolox solution was reacted with 3 mL of FRAP solution, incubated 30 minutes at room temperature and then measured with a UV-Vis spectrophotometer at a wavelength of 594 nm and obtained the equation line y = 0.001x + 0.078 with R^2 = 0.998.

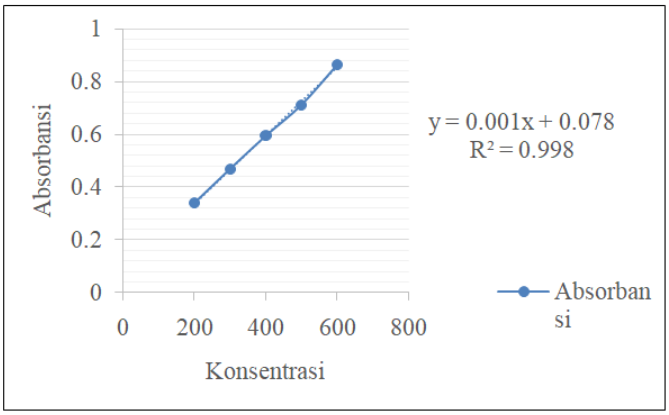

Figure 4. Trolox Calibration Curve

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The TEAC concentration of kedondong juice and the hand body lotion is calculated using the line equation

Tabel 2. Results of antioxidant activity

Testing	Results (mM TE/gram)
*Kedondong*Juice	23,04
Hand body lotionof *kedondong* juice	4,5

The pH of the hand body lotion from kedondong juice was evaluated for 4 weeks and ranged from 7.95 to 7.49.

Tabel 3. pH hand body lotion for 4 weeks

Week to	pH
1	7.95
2	7.96
3	7.53
4	7.49

To evaluate the humidity of hand *body lotion*, 10 volunteers were applied behind the ear and tested with a skin analyzer checker before applying and after applying hand body lotion. The percentage of moisture recovery is calculated by the formula:

$$\% \text{ moisture recovery} = \frac{percent\ moisture\ before\ appling\ HBL}{percent\ moisture\ after\ appling\ HBL} \text{ x } 100\%$$

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Table 4. Percentage of moisture recovery

Volunteers	% moisture recovery			
	week 1	week 2	week 3	week 4
1	25	30.4	33.3	33.3
2	42.9	46.3	50	40
3	39.5	33.3	66.7	26.7
4	20	7.3	11.6	23.4
5	15.4	36.4	43.9	27.7
6	53.9	22.2	31.6	42.5
7	16.7	7.3	22.9	30.4
8	17.8	12.5	28.1	30.4
9	28.2	33.3	36.4	56.8
10	20	9.1	22.4	15.4

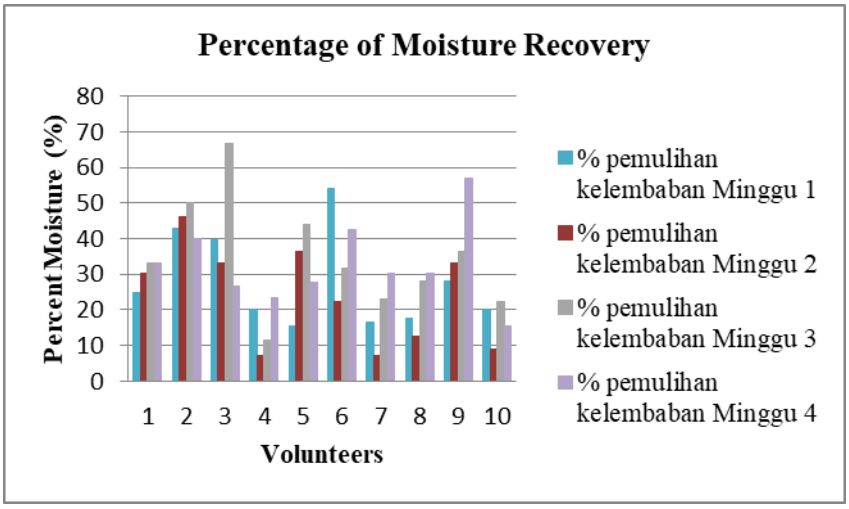

Figure 5. Moisture Recovery Graph

CONCLUSION

The antioxidant capacity of hand body lotion and kedondong juice were 4.5 and 23.04 mM TE/g of the samples, respectively. Evaluation of the quality kedondong juice hand body lotion preparation does not irritate the skin and provides benefits as a moisturizer with a fairly stable pH during the 4-weeks treatment.

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