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Development of Papaya Latex, Papaya Extract (*Carica papaya* L.) and Yam Bean Tuber Extract (*Pachyrrhizus erosus* (L.) Urb.) for Skin Lightening Lotion Based on Tyrosinase Inhibition and Antioxidant Activities

(Pengembangan Getah Pepaya, Ekstrak Pepaya (*Carica papaya* L.) dan Ekstrak Umbi Bengkuang (*Pachyrrhizus erosus* (L.) Urb.) untuk Lotion Pencerah Kulit Berdasar Aktivitas Antioksidan dan Inhibisi Tirosinase)

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Abstract: Whitening or skin lightening products are well known as cosmetic. In this development, papaya latex (*Carica papaya* L.), papaya fruit extract and yam bean tuber extracts (*Pachyrrhizus erosus* (L.) Urb.) were combined for skin lightening body lotion formula. Antioxidant and tyrosinase inhibitory activities were evaluated in the starting materials and the products. Antioxidant activity evaluation was conducted using DPPH free radical scavenging activity method. Tyrosinase inhibitory evaluation was performed based on tyrosinase enzymatic inhibitory reaction to tyrosine as substrate. Papaya latex showed the highest antioxidant activity (32.01%), followed by yam bean tuber extract (27.04%) and papaya extract (26.09%). The tyrosinase inhibitory activities of papaya latex, papaya fruit extract and yam bean tuber extracts were 68.42 %, 36.80% and 52.63 % respectively, as compared to that of arbutin (100%). Based on product appearance, color, odor, pH and viscosity, the product were stable for 128 weeks. The expired date calculation on its antioxidant activity, however, the product was stable for 77 weeks. It is recommended that the product should be stored in non transparent bottle at low temperature or at about 25-28^{o}C.

Keywords: *Carica papaya*, *Pachyrrhizus erosus*, antioxidant, tyrosinase inhibitor, skin lightening.

Abstrak: Produk pemutih dan pencerah kulit sangat dikenal sebagai kosmetik. Pada penelitian ini, kombinasi getah pepaya (*Carica papaya* (L.) Urb.), ekstrak buah papaya, dan ekstrak umbi bengkuang (*Pachyrrhizus erosus* (L.) Urb.) digunakan dalam pengembangan formula *body lotion* pencerah kulit. Bahan baku dan produk *body lotion* dievaluasi aktivitas antioksidan dan inhibisinya pada enzim tirosinase. Aktivitas antioksidan diuji berdasarkan penangkapan radikal bebas DPPH. Evaluasi inhibisi enzim tirosinase dilakukan pada tirosin sebagai substrat. Ekstrak getah pepaya menunjukkan aktivitas antioksidan tertinggi (32,01%), kemudian ekstrak umbi bengkuang (27,04%) dan ekstrak buah papaya (26,09%). Aktivitas inhibisi tirosinase dari getah papaya, ekstrak buah papaya dan ekstrak umbi bengkuang secara berturut-turut masing-masing adalah 68,42%, 36,80% and 52,63% , dibandingkan terhadap arbutin (100%). Dianjurkan bahwa produk disimpan dalam botol tidak transparan pada temperatur rendah atau pada sekitar 25-28^{o}C.

Kata kunci: *Carica papaya*, *Pachyrrhizus erosus*, antioksidan, inhibitor tirosinase, pencerah kulit.

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INTRODUCTION

CURRENTLY, the application of natural products for cosmetics grows rapidly. Development of natural cosmetics is being popular. Yam bean (*Pachirrhyzus erosus* (L.) Urb.) is one of natural products being used for skin lightening. Many Indonesian cosmetic products use Indonesian yam bean, *bengkuang* tuber, and papaya (*Carica papaya* L.) latex extracts. However, very few researches were reported for natural product cosmetics. In modern era, cosmetics become primary need, especially for women who are eager to have good appearance.

Bengkuang tuber (*Pachyrrhizus erosus* L.) is an Indonesian medicinal plants applied as antacid. Furthermore, this tuber starch showed some functional characteristics of gelatinization that is useful in food systems as stabilizing, additives, moisture retainers, and thickener[1]. Yam bean tuber extract has been used for various cosmetic purposes. The main application is for skin masker mixed with talc. Flesh of the tuber is pounded and applied on skin with rashes[2]. The papaya latex extracts are also widely used in cosmetic. Application as soap ingredient, skin cleanser, body lotion are available in Indonesian market[3]. Papaya latex extract application in body lotion has been claimed as a safe natural whitening agent and, is expected to produce skin lightening synergistic effect with yam tuber extract.

In Indonesia there are many papaya plant varieties. Some of them are *pepaya semangka*, *pepaya jinggo*, *pepaya cibinong, pepaya mas*, *pepaya item*, *pepaya thailand*, *pepaya meksiko*, and *pepaya solo*. Papaya transgenic has been developed, however, it is likely that this transgenic plant has not been cultivated and applied in Indonesia[4]. Papaya leaves and latex are also popular in Indonesia, the leaves are used as vegetable, and together with latex used for meat softening and meat tendering[4,5]. The popular papaya is Thailand variety due to its large size, thickness and has pink color appearance[4]. According to patient feelings and blood reports, it showed that *Carica papaya* leaves aqueous extract exhibited potential activity against dengue fever. Furthermore, different parts of papaya leaves can be further used as a strong natural candidate against viral diseases[6]. Papaya latex has been studied for its anthelmintic activity against *Heligmosoraoides polygyrus* in experimentally infected mice. The results suggested a potential role of papaya latex as an anthelmintic against patent intestinal nematodes of mammalian hosts[7].

Papaya latex contains 50% of non water soluble matter which do not show any enzymatic properties. Papain is the major proteolitic constituent of the latex from papaya plant, leaves and fruit. The highest papain content is from young fruit. In market the papain is sold in the form of crude papain, or papain crystal. The crude papain is powdered dried papaya latex and papain crystal is purified form of crude papain. Papain is used for medicine, food (tendering meat), and cosmetics. The papain enzymatic effect is maximum at pH 4.5 to 6.0, and the highest activity is at pH 6.0[8]. Heat, light, and air are the cause of decreasing quality of papaya latex, and the enzymatic activity decreases. Papain accelerates skin replacement process by removing the dead epidermis (stratum corneum), and helps other lightening active substances safely penetrate into the subsurface (endodermis) of the skin.

This paper reports the combination effect of papaya latex and yam bean tuber extracts in its skin lightening effect and to report the stability of the prepared body lotion formula of these combined extracts.

MATERIALS AND METHODS

Materials. Papaya latex was obtained from 2 months old papaya fruit. Papaya extract (thick liquid) and yam bean tuber extract *Pachyrhizus erosus* (L.) Urb. (powder) were obtained from available commercial source. 1,1-diphenyl-2-picryl hydrazil (DPPH), tyrosinase, DL-tyrosine, phosphate buffer pH 6.8, DMSO, methanol, vitamin C, magnesium ascorbyl phosphate and arbutin were purchase from Aldrich. Other chemicals for body lotion formulation are analytical or pharmaceutical grades.

Methods. Antioxidant activity assay. The antioxidant activity of papaya latex, papaya extract, and yam bean tuber extract were measured as free radicals scavenging activities using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to Yen and Chen[9]. Each sample solution in methanol with concentration series of 10, 50, 100, and 200 µg/mL was mixed thoroughly with a one mM methanolic solution of DPPH radicals and left to stand for 30 min in the dark. The absorbance was then measured at λ 517 nm. The following equation was used to calculate the free radical scavenging activity of a sample: Sample scavenging activity (%) = $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control (one mM DPPH solution without sample) and A_1 was the absorbance of DPPH solution in the presence of a sample. Vitamin C, a known antioxidant was used as a reference compound.

Tyrosinase Inhibition Assay. The tyrosinase inhibition assay was conducted according to Shimizu, et al[10]. Similar series concentration of papaya latex, papaya extract, and yam bean tuber extract were

prepared in DMSO. Sample was mixed with 0.3 mg/mL DL-tyrosine and 1 mL of phosphate buffer pH 6.8. After pre-incubation at 37°C for 20 minutes, 1 mg/mL tyrosinase was added. Absorbance was measured at λ 475 nm, before incubation and after incubation at 37°C for 20 minutes. The following equation was used to calculate tyrosinase inhibition activity: [(B-A)/(D-C)]×100%, where A was the absorbance of sample before incubation, B was the absorbance of sample after incubation, D was the absorbance of control (DMSO) before incubation and C was the absorbance of control (DMSO) after incubation. Arbutin, a known tyrosinase inhibitor was used as a reference compound.

Protein content. Protein content in papaya latex, papaya extract, and yam bean tuber extract was determined according to the method in Indonesian National Standard, SNI 01-2891-1992.

Proteolitic enzyme content. Proteolitic enzyme content in the sample was determined according to Kunitz method[11].

Preparation of body lotion. Dipropylene glycol, PEG 1500, and trietanolamin were added into the purified water and heat to 70°C (water phase). Solution of the oil components was prepared (stearic acid, cetyl alcohol, petrolatum, squalane), then add the surfactant (sorbitan mono-oleate), propylparaben, methylparaben, and perfume and heat to 70°C (oil phase). The oil phase was added into the water phase and the preliminary emulsification was carried out. After the particle size uniform using a homogenizer, then the emulsion was de-aerated, strained and cooled.

Body lotion product and stability evaluation. The body lotion products were placed in 6 packaging container and kept in climatic chambers of 25°C, 40°C, 45°C and 50°C. The lotion visual appearance, color, and odor were observed, and antioxidant activities, pH, and viscosities were measured every week. The effect of temperature on reaction rate of the antioxidants were calculated using the Arrhenius equation[12].

$$k = A.e^{-Ea/RT}$$

k = reaction rate constant
A= constant frequency factor
Ea = activation energy
R = gas constant (1,987 x 10-3 kcal/deg mol)
T = temperature (oK)
e = exponential

Antioxidant reaction rate constant (k) was determined from the graph of logaritmic correlation between antioxidant activity with time, to obtain equation Y= bX+a; k = -b × 2.303, where a= intercept; b=slope; Y=log antioxidant activity; X= time (week).

Expiration date (t_{90}) and half life (t_{50}) of the body lotion were calculated based on equation of the line of correlation between log k and 1/T (°Kelvin), Y= bX + a, and then by using the equation of log reaction rate at 25°C was calculated and k value (25°C) was determined.

$$t_{90} = \frac{\log(100/90) \times 2.303}{k(25^{o}C)}$$

$$t_{50} = \frac{\log(100/50) \times 2.303}{k(25^{o}C)}$$

RESULTS AND DISCUSSION

Antioxidant activity. The results of antioxidant activity of papaya latex, papaya extract and yam bean tuber extract is shown in Table 1. Papaya latex showed the highest free radical scavenging activity as compared to papaya extract and yam bean tuber extract at concentration of 500 ppm. However, activity of those three samples were much lower than the vitamin C that has IC_{50} of < 10 ppm. Vitamin C is not stable in a solution form, and therefore it is rarely used as skin lightening agent. Most common used vitamin C derivate is ascorbyl phosphate, but the compound is not soluble in water, methanol nor ethanol, therefore could not be used for comparison. On the other hand, yam bean tuber extract is already known as whitening agent[13], therefore it could be used for comparison to papaya latex and papaya extract.

Protein content and proteolitic activity of papaya extract. Analysis showed that papaya latex has 10.15% protein content and 47.97 unit/mL proteolytic activity. The proteolytic activity indicated that papaya latex could be suggested as an effective agent for skin whitening process. With the proteolitic activity, papaya latex will have exfoliating effect to the epidermal skin that makes skin appears more fresh and white. It is expected that the combination of papaya latex as exfoliating and bengkuang yam

Table 1. Antioxidant activity of papaya latex, papaya extract and yam bean tuber extract at concentration of 500 ppm.

Sample	Inhibition (%)
Papaya latex	32.01
Papaya extract	26.09
Yam bean tuber extract	27.04

extract as a tyrosinase inhibitor will give a sinergistic improvement to the skin whitening effect.

Tyrosinase inhibitor activity. Table 2 shows the tyrosinase inhibitor activity of the papaya latex, papaya extract and yam bean tuber extracts as compared to arbutin as the positive control. These results suggest that all samples have potential to be used as natural source for skin whitening with papaya latex showed the highest activity as tyrosinase inhibitor.

Formulation. Table 3 shows the formula base of the L6 lotion in our experiment[14]. To this formula base, then the active ingredients, combination of yam bean tuber and papaya extracts were added (Table 4). All together, 6 formulas (Formula L1 to L6) were evaluated for lotion characteristics, as showed in Table 5.

In general, an acceptable body lotion is the body lotion which has viscosity of 100-200 poise, and pH 5.5-6.0[15]. The range of specific gravity of the formulas were from 0.9985 to 1.0015. Evaluation results showed that all formulas (L1-L6) showed good appearance. There were no separation, no coagulation, and no significant viscosity decrease. The pHs were also stable. Evaluation on appearance, viscosity, odor, and color, indicated that all formulas were stable and estimated up to 128 weeks. This estimation was based on the Arrhenius accelerated stability test data in our experiment.

Figure 1 showed the relationship between the antioxidant activity of one of the formulas (Formula L3), and length of storage in various temperatures. Antioxidant activity in all formulas slowly decreased to 8th weeks at 25^{o}C. Formulas L1 to L6 showed antioxidant activities of 6.61%, 8.15%, 13.05%, 7.87%, 14.30% and 0%, respectively. Figure 1 shows the correlation curve of formula L3 antioxidant activity (log % inhibition) and length of storage time in climatic chambers. Analysis of antioxidant activities in formula L1-L6 were evaluated. Based on Arrhenius equation formula L3 will be stable at room temperature for 18 months. The highest antioxidant activity was on formula L5 (14.3%) which contains 1% of yam bean tuber extract and 1% of papaya latex, however, this formula has shorter antioxidant stability (9.4 weeks). Formula L3 which contain 1% of yam bean tuber extract and 2% of papaya extract, was the second best in antioxidant activity (13.1%) but has the longest antioxidant stability (77 weeks), therefore this formula is promising for further study and development.

C. papaya is traditionally used to treat skin disorders. Wound healing properties of the latex have been studied. Papaya latex formulated in Carbopol

Table 2. Tyrosinase inhibitor of papaya latex, papaya extract and yam bean tuber extract calculated relative to arbutin.

Sample	Inhibition (%)
Arbutin	100
Papaya latex	68.42
Papaya extract	36.80
Yam bean tuber extract	52.63

Table 3. Formula base of body lotion (L6).

Ingredients	%
Stearic acid	2.0
Cetyl alcohol	1.5
Petrolatum	4.0
Squalane	5.0
Triethylhexanoin	2.0
Sorbitan mono-oleate	2.0
Dipropylene glycol	5.0
PEG 1500	3.0
Trietanolamine	1.0
preservative	0.2
Perfume	0.2
Water	73.1

Table 4. Active ingredients added into Formula base in Table 3.

Formula*	Active ingredient
L1	1% yam bean tuber extract
L2	2% papaya extract
L3	1% yam bean extract+2% papaya extract
L4	1% papaya latex
L5	1% yam bean extract+1% papaya latex
L6	None

*Ingredients of all formula base are the same as in L6 (Table 3), except for the demineralized water for total volume adjustment, which was calculated depend on the amount of active ingredient added.

Table 5. Evaluation result of body lotion formulas.

Formula	Specific gravity	Viscosity (poise)	pH
L1	1.002	177.9	5.78
L2	0.999	176.9	5.79
L3	0.999	177.8	5.80
L4	0.999	177.7	5.84
L5	1.002	178.1	5.82
L6	0.999	177.9	5.81

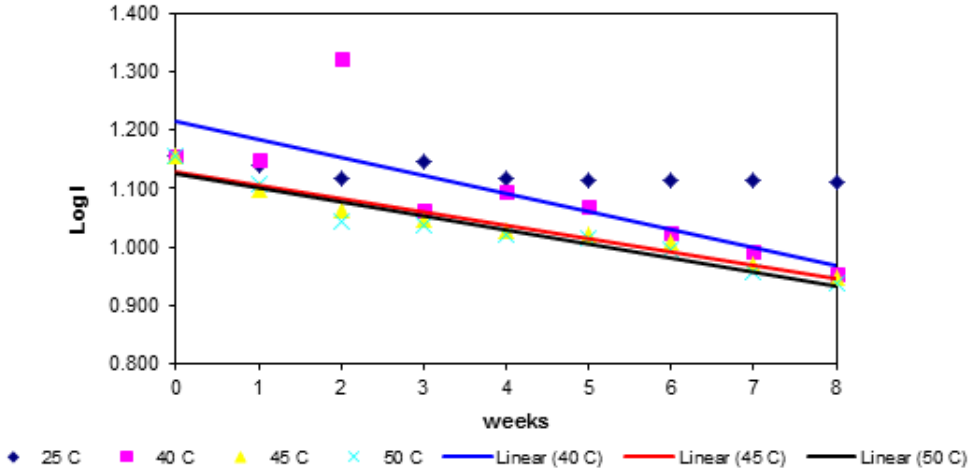

Figure 1. Relationship curve of Formula L3 log antioxidant activity (log I) and length of storage time in various temperatures.

gel was effective in the treatment of skin burns, supporting its traditional use[16]. *In vitro* experiment using various extracts obtained by various solvents showed an anti Gram-positive bacteria *Staphylococcus aureus, Bacillus cereus,* and *Micrococcus luteus* and anti Gram–negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*. These extracts were also active against several fungi, such as, *Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis, Cryptococcus neoformans,* and *Candida kefyr*. These data showed that *C. papaya* extract gave further advantage for cosmetic product formulation[17]. In addition to the content of proteolytic enzyme, the papaya latex also has high content of lipase. The lipolytic activity of *C. papaya* lipase showed its maximum stability at pH 6[8]. The presence of bile acid slightly reduced the *C. papaya* lipase activity in the 4–6 pH range, shifting the optimum lipase activity to pH 7, where the presence of bile had no effect. Lipolysis levels decreased with the pH, but *C. papaya* lipase (CPL) was still more active than porcine pancreatic extract at pH 5 on a relative basis. It is suggested that CPL might be a promising candidate for use as a therapeutic tool on patients with pancreatic exocrine insufficiency[18]. The *C. papaya* latex powder also showed a molluscicidal activity on Lymnaea acuminata (LC_{50} = 8.38 mg/L at 96 hour). The toxicity of this latex has been shown to be time and dose dependent[19].

In the current study, the papaya latex, papaya extract and yam bean tuber extract showed similar antioxidant activity of 32, 26 and 27% respectively at concentration of 500 ppm. Those samples also showed tyrosinase inhibitor activity, as compared to arbutin (a tyrosinase inhibitor). The highest activity was papaya latex (68.42%). Papaya latex has 10.15% protein content and 47.97 unit/mL proteolitic enzyme. The body lotion density was 0.9985 to 1.002 with a viscosity of 177.7 to 178.1 pois; pH was 5.78 to 5.84 and antioxidant activity of 6.1 to 14.3%. Based on product appearance, color, odor, pH and viscosity, the L1 to L5 body lotions were stable for 96 weeks. The expired date calculation based on its antioxidant activity, however, the products were stable for 9.4 to 77 weeks. Formula 3 is suggested as the formula for further study and development because of its antioxidant activity and has longest antioxidant stability.

CONCLUSION

Papaya latex, papaya fruit extract, and yam bean tuber extract show the potency as natural skin lightening agent. This potency was evaluated based on their tyrosinase inhibitory and antioxidant activities. Body lotion formulas were prepared as example in cosmetic application.

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