FORMULATION OF LIQUORICE ROOT EXTRACT (*Glycyrrhiza glabra L.*) AS SKIN WHITENING CREAM

Siti Umrah Noor*, Faridah, Michico
Faculty of Pharmacy Pancasila University, Jakarta 12640
*E-mail: siti_umrahnoor@yahoo.com

ABSTRACT

Liquorice root extract (*Glycyrrhiza glabra* L.) contains glabridin, an isoflavane as inhibitors of tyrosinase. This enzyme is responsible in melanin synthesis. The aim of this research was to determine the tyrosinase inhibition activity of liquorice root extract and to formulate into a cream with a variety of emulsifier agent glyceryl monostearate. Liquorice root was macerated using ethanol 96%, invitro tyrosinase inhibition assay was conducted using kojic acid as positive control in 96-well plate. The physical quality parameters of the cream were also evaluated. The results showed that liquorice root extract inhibits tyrosinase with the IC_{50} 126.75 µg/mL. Creams containing 1.01% liquorice root extract were yellowish white, aromatics odour, soft texture, homogen and no segregation in O/W emulsion type. It also showed plastic thixotropic rheological property, viscosity of (2800±0.00) – (4000±0.00) Ps, spreadability of (3029.72±0.81) – (3531.79±6.15)mm2, droplet size of (60.00±0.00) – (65.12±0.01)µm, pH of (4.55±0.03)–(4.63±0.04) and inhibited tyrosinase 10.14 - 19.30%. It can be concluded that the formula with 0.1% of glyceryl monostearate was the best formula that conforms physical quality test and potentially to be developed as a skin whitening cream.

Keywords: liquorice root extract, glyceryl monostearate, tyrosinase inhibitor, skin whitening cream

INTRODUCTION

Increased production and accumulation of melanin locally leads to a local pigmentation or a black spot on certain parts of the face. The production of melanin mediated by tyrosinase enzymes and was accelerated by the UV light. One of the ways to prevent the production of melanin is to inhibit the activity of tyrosinase.

The concept of "from nature to cosmetic" will produce natural cosmetics. Besides more safe, cosmetics made from natural ingredients have been proven to have a better effectiveness, more health, beauty and eco-friendly.

One of the plants that can inhibit the activity of tyrosinase is liquorice (*Glycyrrhiza glabra* L.). Liquorice contains glycyrrhizin (10-25%), liquiritin, liquiritigenin, isoliquiritigenin, isoliquiretin, glizirhizat, glabrenen acid and glabridin. Glabridin, a phenolic compounds (isoflavan) contained in the root of liquorice and acts as an antioxidant, neuroprotective, anti inflammation, anti eczema, anti pruiritis, and other dermatitis symptoms, and also whitening agent (Damle, 2014).

According to Yokota, et al. (1998), glabridin inhibited tyrosinase activity at 0.1 -1.0 µg/ml concentration without affecting DNA synthesis.

Yamauchi, et al. (2011) also showed that glabridin has stronger activity than kojic acid. Depigmentation effect of glabridin was 15 times better than kojic acid. Glabridin can be effectively extracted from liquorice by using 96.0% ethanol for 240 minutes at temperature below 50°C.

The extract was formulated as a cream (o/w) based on the concentration obtained from the in vitro enzymatic assay. The cream was made using nonionic surfactants polysorbate 80 combined with co-emulgator cetyl alcohol, cetearyl alcohol and glyceryl monostearate. The variation of glyceryl monostearate concentration as co-emulgator was based on the previous research. The addition of 0.5-1% glyceryl monostearate improves the stability and

appearance of the creams. Glyceryl monostearate is “emollient lipophilic thickening agent and stabilizer" that is commonly used in emulsion and cream. It is fatty alcohol group that can improve consistency or act as stiffening agent that increase cream stability.

MATERIALS AND METHODS

Tyrosinase Inhibition Activity: Liquorice root extract (*Glycyrrhiza glabra* L.), kojic acid (Thornhill, Canada), L-DOPA and tyrosinase from Mushroom-lyophilized powder (SIGMA-Aldrich, USA), 0.1 M phosphate buffer pH 6.8, dimethyl sulfoxide1%.

Liquorice whitening cream: Liquorice Root Extract, triple pressed stearic acid (Shanghai FuXin, RRC), cetyl alcohol (BASF, Germany), cetearyl alcohol (Ecogreen, Singapore), anhydrous lanolin (WuXi, RRC), propylene glycol (Dow Chemical Pacific, Canada), parafin liquid (Sonneborn, Netherland), polysorbate 80 (KAO Indonesia Chemical, Indonesia), glyceryl monostearate (Danisco, RRC), methyl and propyl paraben (UENO Fine Chemical, Germany), butylated hidroxy toluene (Sterlitamak Petrochemical Plant, Russia), perfumes, aquadest.

Reagen Sudan III, methylene blue

Equipments and Instruments Microplate reader (BioTek, ELx800), 96-well microtiter plate (Bio-RAD), micropippete (transferpette), kinetic macerator (IKA, RW20), rotary vacuum evaporator (Heidolph, Laborota 4000), water bath (Memmert, WNB400), microbalance (Mettler, MT5), analytical balance (KERN, ABT 120-5DM), homogenizer (IKA, RW20), oven (Memmert, U-110), viscometer (Brookfield RV type), pH meter (HANNA, HI 2211), microscope (Olympus, CH20), centrifugator (Kokusan, K-103N), incubator (Memmert, IN-55), refrigerator (LG).

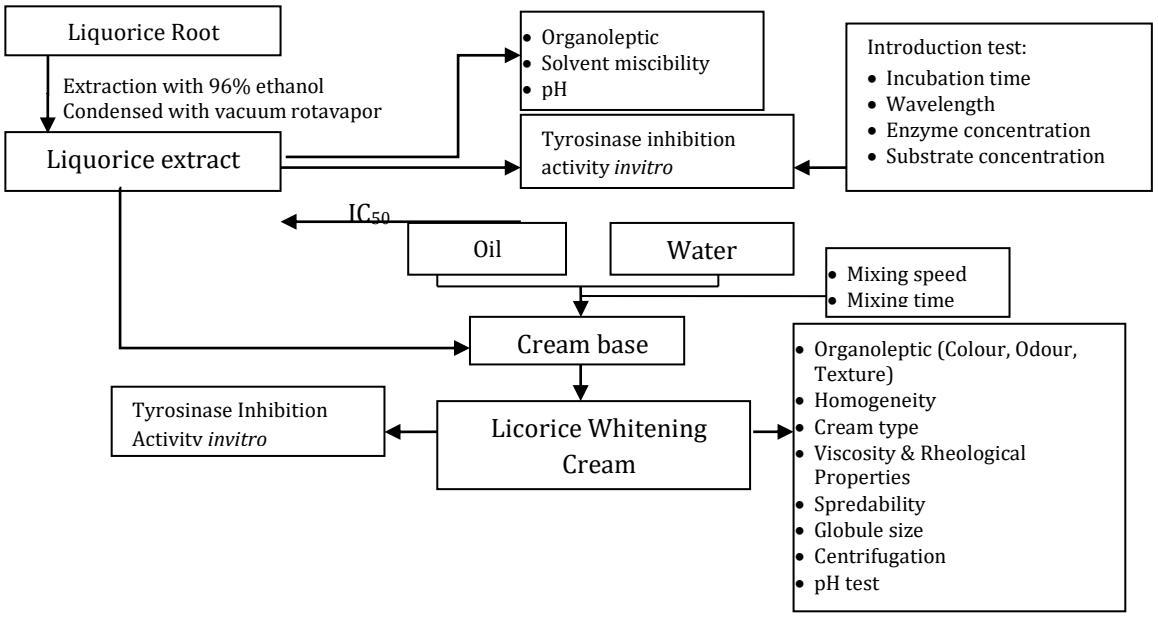


Figure 1– Methods in scheme

Plant material and extraction Plant material was obtained from CV Herbatama (Yogyakarta), was dried by sunlight (indirectly) for 1-3 days and further powdered using a blender and sifted with no. 4/18 mesh. The amount of 500 g liquorice powder was macerated kinetically using 5 L of ethanol (96%) for 4 hours. Remaceration process was done 10 times using 4 L of solvents. The filtrate was evaporated in the rotary vacuum evaporator (temperature of ±

40°C, 180 mmHg pressure, and speed of 60 rpm) to obtain liquorice root ethanol extract.

Inhibition of Tyrosinase Activity invitro This assay was performed using methods as described earlier with modification (Batubara, et.al., 2010). The extract was dissolved in DMSO to a final concentration of 20 mg mL-1. This extract stock solution was then diluted to 25-4000µg mL-1 in 100 mM phosphate buffer (pH 6.8). Kojic acid, the positive control was tested at concentrations of 2.5-50 µg mL-1. In a 96-well plate 80 µL phospate buffer (0.1 M, pH 6.8) was combined with 40 µL L-DOPA (5mM phospate buffer) and 40 µL of sample dilution. After 5 minutes, add 40 µL tyrosinase (310 Units mL-1 in phospate buffer) to each well and further incubated for 20 minutes at 37°C. Optical densities of the wells were then determined at 490 nm. The tyrosinase inhibition activity was calculated according to the following formula:

$$\% \text{ tyrosinase inhibition} = \frac{(B-S)}{B} \times 100\%$$

B : control absorbance – control blank absorbance (B_1-B_0)
S : sample absorbance – sample blank absorbance (S_1-S_0)

Inhibitory activity of the sample was determined by calculating the IC_{50}, namely the concentration in which the sample inhibit tyrosinase activity by 50%

Liquorice cream formulation The cream was made by mixing the oil phase (stearic acid, cetyl alcohol, cetearyl alcohol, lanolyn anhydrous, paraffin liquid, glyceryl monostearate, bht) with the aqueous phase (aquadest 70°c added propylene glycol, polysorbate 80, methyl paraben, propyl paraben) was heated at 70°-75°C (Table 1.).

Table.1 Formula of Cream

Ingredients	FORMULA (%) b/v					
	Blank I	Blank II	Blank III	I	II	III
Liquoorice Root Ethanol Extract	-	-	-	1.01	1.01	1.01
Sodium metabisulfite (to extract)	-	-	-	0,1	0,1	0,1
Stearic acid	2,0	2,0	2,0	2,0	2,0	2,0
Cetyl alcohol	3,0	3,0	3,0	3,0	3,0	3,0
Cetearyl alcohol	6,0	6,0	6,0	6,0	6,0	6,0
Lanolin anhydrous	4,0	4,0	4,0	4,0	4,0	4,0
Paraffin liquid	10,0	10,0	10,0	10,0	10,0	10,0
Glyceryl monostearate	0,1	0,5	0,9	0,1	0,5	0,9
Polysorbate 80	6,0	6,0	6,0	6,0	6,0	6,0
Propylene glycol	15,0	15,0	15,0	15,0	15,0	15,0
Methyl paraben	0,15	0,15	0,15	0,15	0,15	0,15
Propyl paraben	0,05	0,05	0,05	0,05	0,05	0,05
Butyl hydroxy toluene	0,05	0,05	0,05	0,05	0,05	0,05
Perfumes	qs	qs	qs	qs	qs	qs
Aquadest ad	100	100	100	100	100	100

Physical quality parameter Evaluation of the physical quality of cream included: organoleptic (colour, odour, and texture); homogeneity by using a glass object; cream emulsion type with microscopic method; viscosity and rheological properties by using a Brookfield viscometer type RV; spreadability by using a teflon ring; globule size by using an optical microscope; segregation by centrifugation for 5 hours at a speed of 3800 rpm; pH by using pHmeter.

Liquorice cream Inhibition of Tyrosinase Activity in vitro Inhibition of tyrosinase activity was done with a microplate reader. This assay was performed using method as described earlier after the cream was extracted with a solvent and centrifuged for 15 minutes.

Statistical analysis Data of physical parameter assays was analyzed with *ANOVA* (p=0,05), such as viscocity, spreadability, globule size and pH

RESULTS AND DISCUSSION

Tyrosinase Inhibition

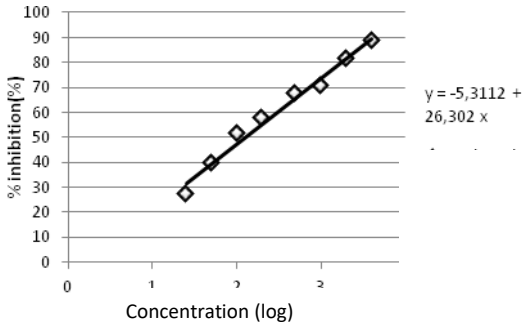


Figure 2. Tyrosinase inhibition activity of liquorice extract

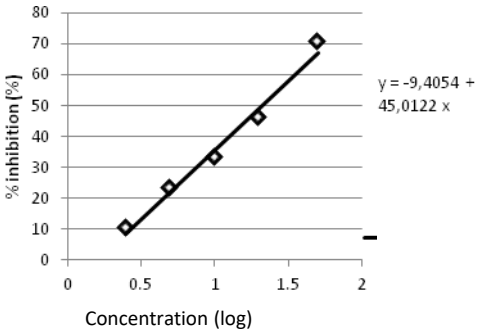
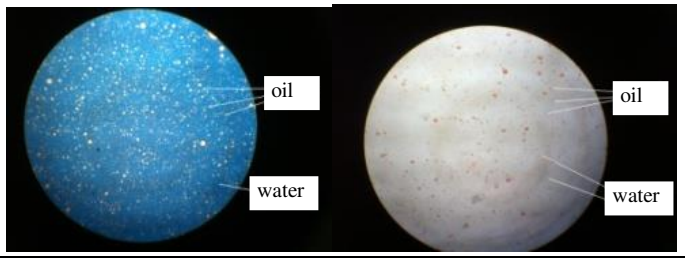
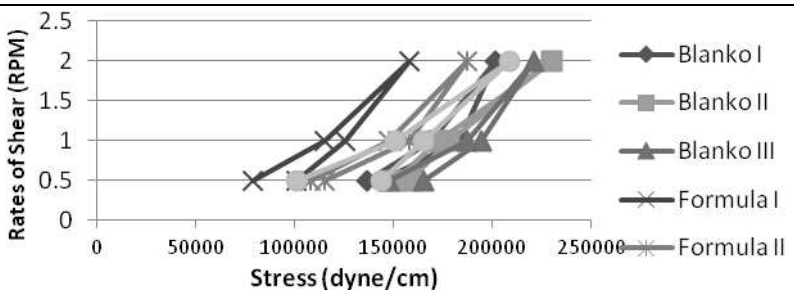


Figure 3. Tyrosinase inhibition activity assay results of kojic acid

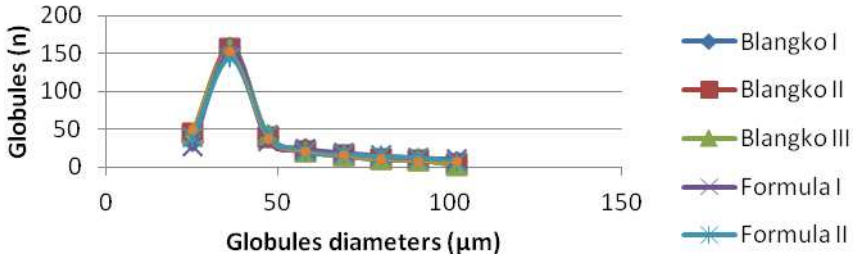
Inhibition of tyrosinase activity in vitro by ethanol extract of the liquorice root and kojic acid (positive control) using enzyme concentration of 310 U/mL, pH 6.8, incubation time of 20 minutes, 5 mM substrate concentration and temperature of 37ºC. Kojic acid chosen as a positive control because of kojic acid is one of the active substances that are commonly used in skin whitening products. Kojic acid inhibits tyrosinase activity by non-competitive inhibition in the oxidation of L-DOPA into the pigment melanin. IC_{50} value of kojic acid obtained in this study was 20.88 $mgmL^{-1}$. When compared with the positive control kojic acid, the liquorice root ethanolic extract showed lower inhibition (IC_{50} 126.76 $mgmL^{-1}$). In this study, the liquorice root ethanolic extract showed a lower activity. This might due to the presence of multi components which disturb the activity.

Physical quality parameter of Cream

Tables 2. Physical quality parameter of cream

Parameters	Formula					
	Blank I	**Blank II**	**Blank III**	**I**	**II**	**III**
Organoleptic	Milky white, aromatic odours, soft textures	Milky white, aromatic odours, soft textures	Milky white, aromatic odours, less soft textures	Yellowish white, Aromatics odours, Soft textures	Yellowish white, Aromatics odours, Soft textures	Yellowish white, Aromatics odours, less Soft textures
Homogeneity	Homogen					
Type of cream	O/W 					
Viscosity (Ps)	4000	4400	4600	2800±0,00	3200±100	4000±50
Yield value(dyne/cm)	83817,27-149957,53					
Rheological Properties	 Rheological : Tyxotrophy plastic					
Spreadability (mm^2)	3053.41	2927.69	2885.98	3531,79±6,15	3394,77±24,95	3029,72±0,81
Globule size (µm)	60.81	58.21	56.67	65.12±0,01	63.72±0,02	60.00±0,00

Distribution Curve



Centrifugation	(-) segregation did not occur					
pH	4.39	4.38	4.35	4.63 ±0,04	4,55±0,03	4.59±0,03
Activity (%)				19.30	10.14	-17.78

The results of physical quality parameter showed that Formula I (0,1% glyceryl monostearate) has soft textures, so that it is convenient to use and easy to spread. It has a higher tyrosinase inhibition activity. The variation of gylceryl monostearate concentration in cream formula related with the softness textures of cream, viscosity spreadbility, globule size, pH and inhibition activity. Higher concentrations of glyceryl monostearate caused the cream less soft, higher visocity, lower spreadability and lower pH value. This is due to the function of glyceryl monostearate to improve its cream consistency. The addition of stearyl alcohol also can improve the texture, add hardness that can be softened with cetyl alcohol that makes the cream remaines soft. Glyceryl monostearate as co-emulsifier can reduce the size of the oil globules and can reinforce the coating film, thus increasing the cream consistency. The o/w cream emulsion type can be determined by the addition of sudan III, which turned into colorless outer phase and red globules inner phase. Addition of methylene blue caused blue outer phase and colorless inner phase.

The viscocity of formulas had a lower value than blank formula. This is due to the addition of liquorice extract which tends to be acidic. The interaction between the extract containing polyphenols and saponins with the base of the cream caused a decrease in viscosity. The ability to spread decreases with increasing concentration of glyceryl monostearate. It has a hight molecular weight with a low degree of spreadability. Most globules obtained in the range of 31-41 µm and decreases with the increasing in diameter. Globule size distribution showed a normal distribution, which can lead to stability of the emulsion according to Stokes law. It can be concluded that the concentration of glyceryl monostearate used can affect the liquorice cream globule size distribution.

The formulas showed no sedimetation after centrifugation for 5 hours 3800 rpm, indicating its stability. Concentration of emulsifier in the cream is enough to form a monomolecular layer on the surface of the oil globules, thus preventing coalescence.

The pH of the formulas was almost similar to the skin's normal pH range (4.5-6.5) and this will prevent skin from irritating and damaging.

Percent of inhibition was decreased with the increasing of glyceryl monostearate concentrations used in cream. The higher concentrations of glyceryl monostearate, the closer film formed and the closer matrix were available. It was more difficult to extract the compounds. High consistency of cream also produced a complex matrix interferring the inhibitory activity. Based on previous research, liquorice extract contained glycosides, saponins, flavonoids and tannins with different stability. The total content of these compounds, especially

total polyphenols and total flavonoids affect the activity of the extract in inhibiting tyrosinase. Cream formulation using crude extract contains constituents that might be able to disrupt the activities. Inhibitory activity of formula III result was negative, this might due to the amount of glyceryl monostearat which is close to 1%.

CONCLUSSION

Liquorice (*Glycyrrhiza glabra* L.) ethanol extract is potential to be developed as whitening agent. It inhibited tyrosinase activity with IC_{50} value of 126.75 ppm. Liquorice ethanol extract 1.01% can be formulated into creams which passed physical quality test. Our study indicated that formula with 0.1% gliceryl monostearate concentration (Formula I) is the best formula among the others.

ACKNOWLEDGEMENT

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