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Skin Brightening Cream Formulation and Tyrosinase Inhibition Assay of Moringa Leaf Extract

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ABSTRACT

Moringa oleifera L. leaves are known to have very high protein content and contain isoflavones, inhibiting tyrosinase activity. The study was conducted to test the tyrosinase inhibitor activity of Moringa leaf extract and to formulate the extract as a skin brightening cream. Research shows that the extract of Moringa leaves has good activity as an inhibitor of tyrosinase and can be developed as a skin brightening cream. The results of the cream formulations showed good cosmetology properties (pH, homogeneity, and spreadibility).

KEYWORDS: Tyrosinase, Moringa oleifera, Moringa

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INTRODUCTION

As heterogeneous polyphenols with a complex structure and color vary from yellow to black, Melanin has an essential role in protecting the skin from exposure to ultraviolet light (UV). (Kwon, 1989; Prota, 1988). In fungi and vertebrates, tyrosinase is known to catalyze the initial steps in melanin formation. (Gillbro, 2011) Unwanted hyperpigmentation often creates severe aesthetic problems, even though Melanin plays an essential role in protecting human skin from UV radiation. This is the basis why research subjects lately are always associated with tyrosinase inhibitory activity. (Chang, 2005).

The abundance of phytochemicals in moringa species allows researchers to explore various compounds that are somewhat unique. This plant family is rich in simple sugars, rhamnose, and is rich in a relatively particular group of compounds called glucosinolates and isothiocyanates. (Bennett, et al., 2003) (Fahey, Zalcmann, & Talalay, 2001). For example, the specific components of the preparation moringa have been reported to have hypotensive activity, anticancer and antibacterial, including 4- (4'-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocy-anate. (Abrams, 1993), 4-(α-L-rhamnopyranosyloxy) benzyl isothiocy-anate (Abuye C, 1999)**,** niazimicin (Akhtar AH, 1995), pterygospermin (Anderson DMW, 1986)**,** benzyl isothiocyanate (Anwar F, 2003)**,** and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (K, 1995)**.** Moringa is also rich in common compounds such as β-carotene or pro-vitamin A. (LJ, 2001)

Tyrosinase inhibition of Moringa leaf extract and its formulation of the extract into a skin brightening cream were the study's objectives. This is based on the absence of research on the tyrosinase inhibitory activity of Moringa extract and its formulation as a skin brightening cream.

MATERIAL AND METHODS

Chemicals and Instrument

Moringa leaves (*Moringa oleifera* L.) were cultivated from Pangkaje'ne District and Islands, South Sulawesi, Indonesia; Ethanol 70% (One Med), Kojic acid, Acetic Acid, H_2SO_4 (Emsure), H_2SO_4 6N (Emsure), Mg (Emsure), HCl (Emsure), 1% FeCL3, Stearic Acid, Glycerin, Sodium Tetraborate, Tyrosinase, Triethanolamine, Distilled Water, Nipagine, and Oleum Rosae. Round bottom flask (Pyrex), 250 ml Erlenmeyer (Pyrex), 250 ml Beaker glass (Pyrex), measuring cup (Pyrex), parchment paper, evaporator (Eyela), test tube (Pyrex), parchment paper, viscometer (Wagtech), universal indicator paper and ph meter (Milwaukee).

Extraction

About 500 grams of Moringa leaves Simplicia powder soaked in 3000 ml of 70% ethanol, then covered and left for two days and every 1X24 hours was stirred. After 2 days of sprinkling, wring it out, wash the dregs with 750 70% ethanol. Then transferred to a closed vessel, remacerate for 2 days, then pour the macerate and filter it. The resulting macerate is then evaporated with a rotary evaporator at a temperature of 50ºC, until a thick extract is produced. The viscous extract was

weighed and the yield of viscous extract was calculated. (Yield = Weight of extract: weight of simplicia X 100%)

Tyrosinase Inhibition Activity

Four test tubes were prepared (A, B, C, D) for tyrosinase inhibitory activity. Each tube was piped about 1.0 ml of L-DOPA 2.5 mm solution and 1,8 ml of phosphate buffer 50 mm solution (pH 6.8) and they were incubated for 10 minutes. After incubation, each tube was added with:

Tube A: 0.1 ml phosphate buffer and 0.1 ml tyrosinase enzyme solution.

Tube B: 0.2 ml of phosphate buffer

Tube C: 0.1 ml sample solution and 0.1 ml tyrosinase enzyme solution

Tube D: 0.1 ml sample solution and 0.1 ml phosphate buffer

The tubes were then incubated for 25 minutes and the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 475 nm. IC_{50} determined the inhibitory activity of the test samples.

Formulation of Skin Brightening Cream

The cream was made in 3 formulas distinguished by the concentration of the ethanol extract of Moringa leaves, each containing ethanol extract of Moringa leaves with varying concentrations, namely 2%, 4%, and 6% 20 g in varying concentrations compositions.

Table 1. Composition of the developed cream

Ingredient	F1*	F2**	F3***	Uses
Moringa extract	2%	4%	6%	Active Singredient
Stearic acid	2g	3g	4g	Emulsifying agent
Gliserine	3g	3g	3g	Humectant
Trietanolamine	0,6g	0,4g	0,8g	Emulsifying agent
Water	qs	qs	qs	Solvent
Nipagine	0,06g	0,06g	0,06g	Preservative agent
Cetyl alcohol	0,6g	0,8g	1g	Stiffening and Emulsifying agent

*F1 = Cream with extract concentrations of 2%; **F2 = Cream with extract concentrations of 4%; ***F3 = Cream with extract concentrations of 6%

Evaluation of Cream

The cosmetological characteristic such as color, odor, homogeneity, pH, spreadability, type of emulsion, and stability was evaluated to determine cream properties. The type of emulsion was determined using the methylene blue dye method. The pH of the cream was observed by a pH meter as described by Ramawanty et al. (Rahmawanty D, 2015) The consistency of the cream was determined by the viscometer. The homogeneity was evaluated using the method described in Farmakope III (Indonesia D. K., 1979). The spreadability of the cream was assessed using a technique described by Garg *et al.* (Garg A, 2002).

RESULTS AND DISCUSSION

Tyrosinase Inhibition Activity

Moringa extract had a higher IC50 value than Kojic acid, as can be seen in Table 3. It was indicated that the extract exhibited lower tyrosinase inhibition activity than the positive control. However, according to previous research, it is known that compounds with tyrosinase inhibitory activity have an IC50 value of less than 100 µg / mL and still have the potential to be Developed as skin whitening or lightening agents. (Cuorto E., 1999) Hence, Moringa extract could be a good candidate as a natural skin brightening agent.

Cosmetological Properties of Cream

The combination of fatty acids and alkalis (stearic acid and TEA) is one way to obtain the emulsifying properties, which are the main character of the vanishing cream base. (R., 1994) (Rowe RC, 2009) Therefore, researchers are careful to select excipients to ensure that the bioactivity of the active compounds in the extract is maintained. The choice of glycerin as a humectant is made by considering its ability to provide moisture to the skin through water absorption from the surrounding environment. Humectants can also help active ingredients cross these layers to reach target cells. The stiffeners chosen were Cetyl alcohol and stearyl alcohol. Cetyl alcohol can give the cream a smooth texture, while stearyl alcohol provides a good consistency. Methylparaben and propylparaben are used as preservatives synergistic because they are first distributed into the water phase, whereas propyl paraben in the oil phase. (Lachman L, 1994).

Table 2. Comparation of Moringa Extract Brightening Cream

Formula	Concentration	Weight (g)	Spreadability (cm^2)	Homogenity	pH
I	2%	10	8,82 cm^2	-	5.5
		20	9,3 cm^2		
		30	9,58 cm^2		
II	4%	10	9,9 cm^2	+	5.5
		20	10,28 cm^2		
		30	11,09 cm^2		
III		10	8,1 cm^2	-	5.5
	6%	20	8,6 cm^2		
		30	8,88 cm^2		

Table 3. IC_{50} value of Moringa Extract Tyrosinase Inhibition Activity

Tested sample	IC50 (µg/mL) ± SD	Reference
Moringa Extract	92.374 ± 8.11	Present work
Kojic acid	65.031 ± 2.44	Present work

The spread of the cream formulated is shown in Table 2. Moreover, according to SNI (Indonesia S. N., 1998), formulations for application to the skin must have a pH close to skin pH, namely 4.5-6.5 to prevent skin irritation. The cream should also have a good consistency as a topical cosmetic. (Mitzui T, 1997) The pH of the cream and the homogeneity of the formulations have met the requirements, as shown in Table 2. Therefore, it can be said that the cream formulas have good cosmetological properties.

CONCLUSION

Based on the results of this study, it can be concluded that the three formulations of *Moringa oleifera* L. leaf ethanol extract cream obtained from the Pangkajene district can produce a cream that has good stability, which has a good shape, odor, pH, dispersibility, good homogeneity and stability in the type of oil-in-water emulsion cream. Moringa extract has tyrosinase inhibitor activity less than 100 µg/mL which means a promising candidate as a natural skin brightening agent.

REFERENCES

I. Abrams, B. D.-P. (1993). A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. *Journal of acquired immune deficiency syndromes*, 949-958.

II. Abuye C, A. O. (1999). Familial tendency and dietary association of goitre in Gamo-Gofa, Ethiopia. *East African Medical Journal*, 447-451.

III. Akhtar AH, K. A. (1995). Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants of Pakistan in aspirin-ulcerated rats. *Journal of Ethnopharmacology*, 1-6.

IV. Anderson DMW, P. B. (1986). The gum exudates from Chloroxylon swietenia, Sclerocarya caffra, Azadirachta indica and Moringa oleifera. *Phytochemistry*, 247-249.

V. Anwar F, a. M. (2003). Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. *Journal of Agricultural and Food Chemistry*, 6558-6563.

VI. Bennett, R. N., Mellon, F. A., Foidl, N., Pratt, J. H., Dupont, M. S., Perkins, L., & Kroon, P. A. (2003). Profiling Glucosinolates and Phenolics in Vegetative and Reproductive Tissues of the Multi-Purpose Trees Moringa oleifera L. (Horseradish Tree) and Moringa stenopetala L. *Journal of Agricultural and Food Chemistry*, 3546-3553.

VII. Bursala, E., & Köksal, E. (2010). Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (Rhus coriaria L.). *Food Research International, 44*(7), 2217-2221.

VIII. Chabani, S., Lavaud, C., Benkhaled, M., Harakat, D., Long, C., & Haba, H. (2016). Three new oleanane-type triterpene saponins from Atractylis flava. *Phytochemistry Letters, 15*, 88-93. doi:10.1016/j.phytol.2015.11.017

IX. Chang, T.-S. D.-Y.-C. (2005). Identifying 6,7,4′-Trihydroxyisoflavone as a Potent Tyrosinase Inhibitor. *Bioscience, Biotechnology, and Biochemistry*, 1999-2001.

X. Cuorto E., K. C. (1999). Inhibitors of Mammalian Melanocyte Tyrosinase: In Vitro Comparisons of Alkyl Esters of Gentisic Acid with other Putative Inhibitors. *Biochem Pharmacol*, 57:663–72.

XI. Daniele, C., Dahamna, S., Firuzi, O., Sekfali, N., Saso, L., & Mazzanti, G. (2005). Atractylis gummifera L. poisoning: an ethnopharmacological review. *Journal of Ethnopharmacology, 97*(2).

XII. Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 5-51.

XIII. Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., & Abdelly, C. (2008).

Phenolic composition of Cynara cardunculus L. organs, and their biological activities. *Comptes*

XIV. *Rendus Biologies, 331*(5), 372-379. doi:10.1016/j.crvi.2008.02.008.

XV. Garg A, A. D. (2002). Spreading of Semisolid Formulations. *Pharmaceutical of Technology*, 84-105.

XVI. Gillbro, J. M. (2011). The melanogenesis and mechanisms of skin-lightening agents - existing and new approaches. *International Journal of Cosmetic Science*, 210-221.

XVII. Indonesia, D. K. (1979). *Farmakope Indonesia III* . Jakarta: Direktorat Jendral Pengawasan Obat dan Makanan.

XVIII. Indonesia, S. N. (1998). *Skin Whitening Cream.* Badan Standardisasi Indonesia.

XIX. Jiangning, G., Xinchu, W., Hou, W., Qinghu, L., & Kaishun, B. (2005). Antioxidants from a Chinese medicinal herb – Psoralea corylifolia L. *Food Chemistry, 91*(2), 287-292.

XX. K, A. (1995). The major constituents of the acetone fraction of Ethiopian Moringa stenopetala leaves. *Mansoura Journal of Pharmacological Science*, 55-64.

XXI. Kwon, B. S. (1989). Isolation, Chromosomal Mapping, and Expression of the Mouse Tyrosinase Gene. *Journal of Investigative Dermatology*, 589-594.

XXII. Lachman L, L. H. (1994). *Teori dan Praktek Farmasi Industri. 3rd ed.* Jakarta: Universitas Indonesia.

XXIII. LJ, F. (2001). *The Miracle Tree: The Multiple Attributes of Moringa*. Dakar: Church World Service.

XXIV. Miguel, M. G. (2009). Antioxidant activity of medicinal and aromatic plants. A review. Antioxidants and Oxidation. *Flavour and Fragrance Journal, 25*(5), 291-312. doi:10.1002/ffj.1961

XXV. Mitzui T, e. (1997). *New Cosmetic Science. 1st ed.* Elsevier.

XXVI. Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology, 26*(2), 211-219.

XXVII. Prota, G. (1988). Progress in the chemistry of melanins and related metabolites. *Medicinal Research Reviews*, 525-556.

XXVIII. R., V. (1994). *Buku Pelajaran Teknologi Farmasi 5th ed.* Yogyakarta: Gadjah Mada University Press.

XXIX. Raghuveer, C., & Tandon, R. (2009). Consumption of functional food and our health concerns. *Pakistan Journal of Physiology, 5*(1), 76-83.

XXX. Rahmawanty D, Y. N. (2015). Formulation and evaluation of peel off face mask containing quersetin with various in the concentration of gelatin and glycerin. *Media Farmasi*, 17-32.

XXXI. Ramamoorthy, P., & Bono, A. (2007). Antioxidant activity, total phenolic and flavonoid content of Morinda citrifolia fruit extracts from various extraction processes. *Journal of Engineering Science and Technolog, 2*(1), 70-80.

XXXII. Rhaffari, L. E., & Abdelhamid, Z. (2002). Pratique de la phytothérapie dans le sud-est du Maroc (Tafilalet) : un savoir empirique pour une pharmacopée rénovée. In J. Fleurentin, J. Pelt, & M. G., *In Des sources du Savoir aux médicaments du futur* (pp. 293–318). Paris: IRD.

XXXIII. Rowe RC, S. P. (2009). *Handbook of Pharmaceutical Excipients. 6th ed.* Grayslake: Pharmaceutical Press.

XXXIV. Spritz, R. A. (1994). Genetic Disorders of Pigmentation. In R. A. Spritz, *Advances in Human Genetics* (pp. 1-45).

XXXV. Yong-guang, B., Ding-long, Y., XIAO-jun, H., Yu-min, L., & Min-xia, H. (2012). Study on Ultrasonic-assisted Extraction of Polysaccharide of Atractylis Macroceohala Koidz of Experiment*. *Energy Procedia, 17B*, 1778-1785.