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FORMULATION OF MORINGA SOAP AS AN ANTIOXIDANT TO PROTECT FREE RADICALS ON THE SKIN

Maria Hilaria[1*], Priska Ernestina Tenda[1], Yorida Febry Maakh[1]

[1]Health Polytechnic of Kupang, Piet A Tallo Street , Kupang, 85111

*E-mail: mariahilaria023@gmail.com

ABSTRACT

The preparation of bath bar soap can be used to clean the skin from dirt, and can be useful as antioxidant that can protect the skin from free radicals. This study aims to determine whether a solid soap formulation from ethanol extract of Moringa leaves could meet the physical characteristics of the soap and the test of antioxidant activity. The research design used was experimental laboratory. Moringa leaves ethanol extract obtained by soxhletation method with 96% ethanol solvent. The extract then formulated in 5 bar soap formulations with various concentrations of Moringa extract were FI (0g); FII (1g); FIII (2g); FIV (3g); FV(4g). Bath bar soap would be tested for the characterization of the soap, namely organoleptic, water content, amount of free alkali, pH and ethanol insoluble material according to the requirements of the Indonesian National Standard 3532:2021. Antioxidant activity of bath bar soap would be tested by the DPPH reduction method. The results of the physical quality showed that the Moringa extract bar soap had organoleptic of solid, brownish green in color, had uniform homogeneity, the pH value was qualified the requirements of SNI, the water content was qualified the requirements of SNI, the stability of the FI-FIV foam was qualified the requirements, the ethanol insoluble material FI, FII, FIV were not qualified the requirements. Requirements, for testing the free alkali content of FIII was qualified the requirements of SNI. The bath bar soap of moringa extract showed an weak antioxidant activity.

Keywords: *Bar soap, Moringa extract, Antioxidant*

INTRODUCTION

The skin function is to protect the inside of the body from physical and mechanical disturbances, heat, cold, germs and bacteria. Protection and treatment for the skin is needed because of the function of the skin as a protector of tissues and organs, one of which is by cosmetics usage (1).Cosmetics are preparations used on the outside of the human body to clean, give a good scent, change appearance and/or improve body odor or protect or nourish the body. One of the cosmetics used to treat the skin is soap. Bath soap can clean, treat and protect the skin, clean dirt, and used to nourish healthy skin from free radicals as antioxidants and anti-bacterials (2).

Antioxidants have the ability to inhibit or prevent the oxidation of a molecules or compounds (3). Antioxidants help protect the body from cell damage caused by free radicals, slowing down the aging process by damaged body cells regeneration, so they are suitable for application in cosmetic preparations. The effect of free radicals on the skin is characterized by skin wrinkled rapidly and black spots formation. This can be overcome with antioxidants, although in fact the human body is capable to synthesizing various antioxidant compounds on its own, if the natural antioxidant defense ability, it can cause disease or skin disorders, therefore additional antioxidants are needed from outside to protect the skin from free radical hazards (4). Antioxidants commonly used for the bar bath production are synthetic antioxidants such as butylhydroxytoluene (BHT), which are unsafe for the skin if used excessively, so in this study antioxidants used are from natural ingredients, namely the Moringa plant which also has anti-bacterial properties (2,5).

Moringa leaves contain bioactive compounds. Compounds contained in Moringa is functionate as antioxidants as well as inhibit the growth of bacteria and fungi. Moringa is rich in phenolics which can inhibit oxidation. Moringa leaves contain 11 phenolic acids (galic acid,

caffeic acid, chlorogenic acid, o-coumaric acid, p-coumaric acid, ellagic acid, gentisic acid, synapic acid, and citric acid), flavonoids (especially flavonols and glycosides: quercetin, rhamnetin, campferol, apigenin, and miricetin), and its derivatives (coumaroylquinic acid and its isomers, feruloylquinic and caffeoylquinic). In addition, Moringa leaves are rich in amino acids, minerals, vitamins, and beta-carotene (6). This study aimed to obtained quality ethanol extract of moringa and can be made into a bar soap that qualified the characteristics of a bath bar soap and has antioxidant activity.

RESEARCH METHODS

The type of research conducted was laboratory experimental. This research was carried out in the pharmaceutical laboratory, phytochemical laboratory and pharmaceutical technology laboratory at Kupang Health Polytechnic of Ministry of Health. The research was conducted from January to November 2022. The equipments used were UV-Vis spectrophotometer (himadzu type 1700), pH meter, vacuum rotary evaporator (Eyela type N-1000), analytical balance (Type EW-220-3NM), oven (Memert), hotplate, blender, test tube, glassware. Research materials used were a sample that taken from the Moringa leaves in the Belo sub-district, Kupang. Other materials used were olive Oil, VCO, technical Cocomid DEA, NaOH p.a. (Merck), technical stearic acid, Glycerin, Sucrose, Ethanol 95% , Chloroform, HCl p.a, $FeCl_3$,p.a., CH_3COOH p.a, Concentrated H_2SO_4 ,p.a., Mayer's reagent, Dragendorf's reagent, Bouchardat's reagent, Standard Buffer Solution pH 4.7 and 10.

Moringa Leaves Ethanol Extract Making

Moringa leaves were determined at Jatinangor Herbarium, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. Moringa leaves were made into simplicia until a simplicia powder was obtained, then 70 grams of simplicia were taken and extracted using a soxhlet method with 96% ethanol as solvent. The liquid extract obtained was concentrated using a vacuum rotary evaporator and evaporated in a water bath at temperature of 50 °C. Extract resulted was weighed to calculated its yield percentage (7) (Equation 1). Phytochemical screening was carried out against the extract, namely testing for alkaloids, flavonoids, tannins and polyphenols, saponins, steroids and terpenoids.

Yield Percent Formula: $\frac{weigh\ \ of\ dried\ extract}{weight\ of\ simplicia}\ x\ 100\%$ ……(Equation 1)

Bath Bar Soap from Moringa leaves Ethanol Extract Making

Moringa soap was made by oil phases such as stearic acid, VCO and olive oil was melted at temperature of 60 °C, then 30% NaOH was added until saponification reaction occurs. The mixtures was added with Cocomid DEA until it was homogeneous. The water phase, namely sucrose and NaCl, was dissolved and then glycerin was added. Water phase was put into the oil phase, then stirred until homogeneous. The temperature was lowered to 30 °C. The extract which has been dissolved in water was added. Pour the soap into the mold and left it for 24 hours (8).

Table 1. Bath Bar Soap of Moringa Leaves Ethanol Extract Formulations (2).

Ingredients	Formula I(g)	Formula II (g)	Formula III (g)	Formula IV (g)	Formula V (g)
Moringa leaves ethanol extract	0	1	2	3	4
VCO	65	65	64	64	63
Olive oil	25	24	24	23	23
NaOH 30%	54	54	54	54	54
Stearic acid	9	9	9	9	9
Aquadest	10	10	10	10	10
Cocoamid DEA	25	25	25	25	25
Glycerin	7	7	7	7	7
Sucrose	12	12	12	12	12
NaCl	0,2	0,2	0,2	0,2	0,2
Total Weight	207,2	207,2	207,2	207,2	207,2

Bath Bar Soap of Moringa Ethanol Extract Characterization Test

The purpose of the soap characterization test was to ensure that the soap to be used qualified the physical requirements of the preparation. The tests carried out were organoleptic, pH, homogeneity, water content, insoluble ethanol matter, free alkali and foam stability test.

The organoleptic test carried out was in parameter of shape, smell and color (9). The pH test was carried out using a pH meter by measured 1 g ± 0.05 of soap in a 1 L volumetric flask (10). The homogeneity test was carried out by applying soap to a transparent glass to observed wether there were granules in the soap or not (11).

Water content test (10) was carried out by a petri dish that had been dried in an oven (105 ± 2) °C was weighed (bo); 5 ± 0.005 soap in a petri dish was weighed (b1); Heat in the oven at temperature of 105 ± 2) °C and cooled down to room temperature then weighed (b2), repeat this procedure until the weight was constant. Water content was calculated using the formula below:

Water Content: $\frac{b1-b2}{b1-b0}\ x\ 100\%$ ..... (Equation 2)

Ethanol insoluble matter test (10) was carried out by (5 ± 0.05) grams of soap was weighed in an Erlenmeyer with a sharpening lid, with 200 mL of freshly boiled ethanol, the vertical cooler was connected, then heating it over a water bath until the soap was completely dissolved; The filter paper or cup was dried in the oven at temperature of (103 ± 2) °C for 30 minutes; Cooled it down in a desiccator, then weighed until the weight was constant (b0); When the soap was completely dissolved, pour the soap solution into a filter paper or cup.

The solution was protected from carbon dioxide and acid during the process by covering it with an Erlenmeyer lid or a watch glass; The material that did not dissolve in Erlenmeyer was washed with hot neutral ethanol; The residue was washed on filter paper or dishes with hot neutral ethanol until soap-free; The filter paper or cup was dried with the residue in an oven at temperature of (103 ± 2) °C for 3 hours; Cooled it down in a desiccator, then weighed until constant weight was obtained (b2). Ethanol insoluble materials then calculated using formula below:

Insoluble-ethanol materials = $\frac{b2-b0}{b1}\ x\ 100\%$..... (Equation 3).

Determination of free Alkali (10) was calculated as NaOH or free fatty acid (calculated as oleic acid). Principle: The filtrate of the insoluble material in alcohol was titrated with a standard acid solution if the phenolphthalein indicator turns out to be an alkaline solution or

titrated with an alkaline standard solution if the phenolphthalein indicator turns out the solution was acidic. Calculated to NaOH if it's alkaline or to be oleic acid if it's acid.

Free alkali: $\frac{40\ X\ V\ X\ N}{b}\ x\ 100\%$..... (Equation 4)

Free fatty acids: $\frac{282\ X\ V\ X\ N}{b}\ x\ 100\%$..... (Equation 5)

Description: Alkali free/fatty acids free in % mass fraction, V: Volume of HCl/KOH used in mL. N: Normality of HCl/KOH used, b: Weight of the sample in mg, 40: Equivalent weight of NaOH and 282: Equivalent weight of moleic acid.

Foam stability test used a method that referred to Handayani (12) in Hasibuan (13). The foam stability test was carried out using the cylinder shake method. 1 g of soap was added with 9 ml of water and then put it into a test tube. The reaction tube was shaken for 30 seconds using a vortex and let it stand for 1 hour. Next, the height of the soap foam was measured.

Antioxidant Activity Test of Moringa Soap

Antioxidant activity test was carried out using method that referred to William et al. (1995) in Hasibuan (13). 0.5 g of solid soap sample was diluted with 5 mL of alcohol and left for 24 hours in a dark room. A total of 1.3 mL of the extract was taken and 5 mL of DPPH solution was added which was prepared by dissolving 0.0001 g of DPPH in 100 mL of alcohol. The solution was incubated for 30 minutes, and then its absorbance was measured at a wavelength of 517 nm. The control that was used was DPPH blank whose absorbance was measured at a wavelength of 517 nm.

Research design

The design used was a single factor complete randomized design with three replications. The factor studied was the weight (g) of Moringa extract in the solid soap formulation.

RESULTS AND DISCUSSION

Extraction of Moringa Leaves

Plant determination aimed to checked the truth or authenticity of the plants used. The results of the determination showed that the moringa plant used in the study was *Moringa oleifera* Lam according to letter No.40/HB/02/2022. Fresh moringa leaves (*Moringa oleifera* Lam), sorted, dried, mashed and then sieved to obtained dry moringa leaves powder with yield percentage of 9.73% w/w ± 0.42. The dried moringa leaves powder was then soxhletated using ethanol as solvent to obtained an extract with yield percentage of 16.81% w/w ± 0.52.Extraction method selection by soxletation was based on the results of research on the total flavonoid content and antioxidant activity tests of *Moringa oleifera* Lam leaves extract by Susanty et al., (7) which had the largest flavonoid content of 245.771 mg/L. Phytochemical screening was carried out to determined the presence or absence of secondary metabolites in moringa leaves extract produced.

Table 2. Phytochemical Screening of Moringa Leaves Ethanol Extract Result

Compounds Group	Reagent	Result	Description
Alkaloids	Mayer	Orange precipitate (white)	+++
	Wagner	Brown precipitate	+++
	Dragendorf	White colour	+++
Flavonoids	Mg + HCl Pekat +etanol	Red colour	+++
Saponins	Sampel + air	Stable foam formed	++
Steroid	Libermann-Burchard	Bluish purple/green colour	++
Tannin	$FeCl_3$ 1%	Blackish purple colour	+++

(Source: laboratory primary data, 2022)
Description: - : not contained; ++: contained weakly. +++: contained strongly

Data in table 2 showed that Moringa leaves ethanol extract contained alkaloids, flavonoids, saponins, tannins and terpenoids. The results of this test are the same as the research conducted by Rahmawati (14). Flavanoids in the ethanol exctract of Moringa leaves are thought to function as natural antioxidants with a mechanism of action as reducing coumpounds (15).

Moringa Ethanol Extract Soap Making

Ethanol extract of Moringa leaves was formulated into a bar soap preparation. Bar soap was chosen because it was most widely used by the community to clean the skin from dirt (16). The soap was formulated into 5 preparations with different extract concentrations in each formula for 3 replications. Based on research from Windi Eka Putri in 2018 which examined the addition of moringa extract to transparent soap. This study used moringa extract using 70% ethanol as solvent and extracted by the maceration method (2). The extract was formulated into transparent soap with several ingredients. The results of the study concluded that the addition of moringa extract greatly affected the quality of transparent soap including color, scent, foaming ability and pH.

Moringa Ethanol Extract Soap Characterization Test

Bath bar body soap test was carried out with the aim of seeing whether the preparation of bath bar soap that was made qualified the requirements in accordance with the Indonesian National Standard number 3532 year: 2021 concerning bath soap and several other requirements. The tests that was conducted included organoleptic, pH, homogeneity, weight uniformity, foam stability, water content, ethanol insoluble matter and free alkali (Table 3).

Organoleptic testing was carried out at room temperature, the result showed that the five formulas had the same form, namely solid dosage form, had the same aroma, which is typical of olive oil. The bar soap produced had a different color depending on the concentration of the extract added. The higher the concentration of Moringa leaves extract added, the color of the solid soap produced was also darker, namely blackish green color. Based on the organoleptic test, the solid soap of Moringa leaves extract qualified the organoleptic evaluation test, during a storage period of 3 weeks stored at room temperature.The pH value determined the appropriateness of soap for use as a bath soap. The pH value of the solid soap produced could be seen in table 3. The pH test conducted in this study aimed to determine whether the pH of bar soap produced affects the irritating properties of the skin (17). According to Rusli, (2018) a very high or very low pH value of soap can increased the absorption power of the skin thereby allowing the skin to experienced irritation (1). The standard pH value for bar soap based on INS number 3532: 2021 was 6 – 11, so that the bar soap produced qualified the INS requirements. The pH of bar soap produced showed a relatively alkaline pH. Basically solid bath soap that was made from fat and alkali had a pH between 8-10, this was because bath soap

that was produced from fat and alkali would not be able to reach a normal pH (pH 7). Bath soap that had a pH of 8-10 was not a place for bacteria to grow. The large amount of alkali in soap was due to the presence of alkali which did not react with fatty acids in the saponification process. A higher pH value made the soap easily foamed because it was alkaline (18)

Table 3. Test Results of Moringa Soap Ethanol Extract

Test	FORMULA I	FORMULA II	FORMULA III	FORMULA IV	FORMULA V
Organoleptic					
Consistency	Solid	Solid	Solid	Solid	Solid
Colour	YW	BG	BG	BG	LG
Aroma	OO	OO	OO	OO	OO
pH	8,72 ± 0,06	8,87± 0,04	8,43± 0,04	8,51± 0,03	8,62 ± 0,04
Homogenity	H	H	H	H	H
Weight uniformity	17,44±0,33	17,65±0,21	17,35±0,26	17,63±0,24	17,54±0,07
Foam height (mm)					
Beginning (0 mnt)	19,30 ± 1,15	21,00±2,52	20,00± 2,89	19,00±2,00	18,00±3,79
Ending (60 mnt)	13,30± 3,25	12,00±1,53	16,00±1.00	13,00±1,53	11,00±0,58
Foam stability	69±0,12	56±0,01	80±0,11	67±0,07	60±0,13
Water content	15,69± 0,81	15,60± 0,49	16,40± 1,27	16,40± 0,65	17,20± 0,71
Ethanol insoluble matter	6,80±6,51	1,60±1,13	17,13±11,67	10,73±8,57	13,00±8,13
Free alkali	0,017±0,02	0,0925±0,02	0,1163±0,01	0,1189±0,01	0,1401±0,01

(Source: primary data, 2022)
Description: YW : Yellowish white; BG : Brownish green; LG : Blackish green; OO :Typical scent of olive oil; H : Homogeneous

Homogeneity test was carried out to found out whether there were granules in the bar soap or not. This test was carried out by looking at the color uniformity of the soap mixed with Moringa leaves extract. If there are granules and the color was not uniform, then the soap was said not homogeneous (11). The results obtained were that there were no granules present in the soap. This showed that formula 1-5 were homogeneously dispersed. Differences in the concentration of Moringa leaves extract did not affect the results of the soap homogeneity test.

The results of the foam height test showed that formula 1 to formula 5 Moringa leaves extract bar soap had a foam height according to the requirements of 13-220 mm. It could be concluded that it had abundant foam which was effective in cleansing the skin and spreading the active substance of Moringa leaves extract. So that it could be more effective in cleansing the skin. Formula 5 did not qualified the requirements because when shaken there was foam height decreased below the predetermined requirements (8).

The foam stability test aimed to determine the stability of the foam produced by bar soap preparations with the addition of surfactant and foam stabilizer, namely comid DEA. In addition to comid DEA, the addition of stearic acid could also stabilized the foam reluted (19). Foam measurements were carried out more related to psychological perceptions of soap being able to clean properly if it produced a lot of foam (20). Bath soap was said to had good stability if it produced a stable foam of between 60-70% within 5 minutes. More than 70% could still be said qualified the requirements (21). The foam stability test results for formulas 1, 3, 4 and 5 qualified the requirements, namely between 60-80%, except for formula 2, which was 56%.

The results of the water content test showed that the Moringa leaves extract bar soap had a water content that varied from 15.69% - 17.20% w/v. The water content of the test results was still within the recommended value of 10-20% (22) and was in accordance with the water content quality requirements based on INS no. 3532: 2021, which was a maximum water content was 23% mass fraction. The water content in bar bath soap showed the amount of water present in the soap. Soap with a high water content would experience shrinkage faster and the soap would became hard when used. So that it became less comfortable when used, while soap that contained a little water content could increased its shelf life. However, the duration of soap storage could affect the hardness of the soap because the water content in the soap evaporated more and more (23). Therefore, the water content of bath bar body soap products greatly determined the characteristics of the soap stored during sale and when it was used by the public.

Ethanol insoluble materials test aimed to identified impurities such as sodium silicate, sodium phosphate, sodium carbonate and materials added in small amounts to soap such as bleach and florescent materials. Formulas III and V did not qualified the requirements for ethanol insoluble materials test. The requirements for ethanol insoluble materials test according to INS 3532: 2021 were maximum of 10%. So, bar soap that qualified the requirements were Formulas I, II, and Formula IV. This may be caused by alkaline impurities used to produced the soap (24).

Free alkali content test of bar bath soap was a measurement of the excessed alkali in the soap or it did not saponified or did not react with fatty acids. The high percentage of free alkali value indicated that the bar soap produced can caused irritation to the skin. The results of free alkali test in bath bar soap were presented in Table 3. Based on the results of the free alkali content test, FII (formula 2) qualified the requirements by INS 3532: 2021. Maximum free alkali content of bar soap was 0.1% (10). The pH value will increased with increasing alkalinity and decreased with increasing acidity, so that the higher the pH value of the soap on the Moringa extract, the higher the alkali content contained in the soap.

Antioxidant activity

Based on the results of the study, it was found that the addition of ethanol extract from Moringa leaves had a significant effect on antioxidant activity (Formula II-V) which was greater than without the addition of ethanol extract of Moringa leaves (FI). Figure 1 shows the IC_{50} value of Formula 1 is higher than Formula II-V. The higher the IC_{50} value the lower the antioxidant activity. Formula II – V showed relatively weak antioxidant activity with an IC_{50} value of 181,27 to 208,16. Formula V produced the largest antioxidant activity compared to thr other formulas, namely 181,27, although its activity was still relatively low.

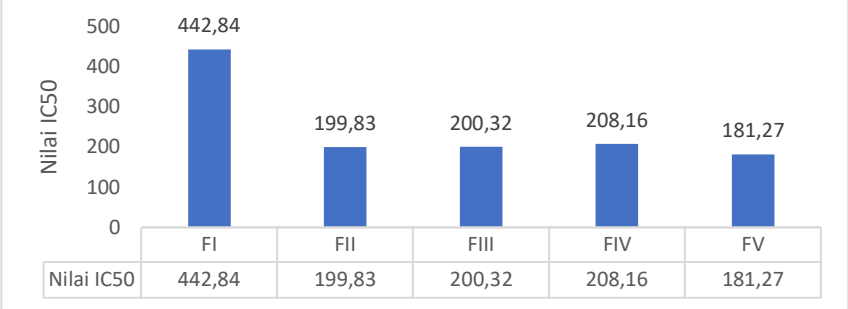

Figure 1. IC_{50} value of antioxidant activity test of Moringa leaf bath bar soap.

CONCLUSION

Based on the research results, it can be concluded that the ethanol extract of Moringa leaves contains alkaloids, flavonoids, saponins, tannins and terpenoids. Ethanol extract can be made into bath bar soap preparations. Based on the characterization tests, formula 1 to 5 qualified the test requirements for organoleptic, homogeneity, pH and water content. For the foam stability test, formula 1, 2, 4 and 5 qualified the requirements, in ethanol insoluble materials test, bar soap that qualified the requirements were formulas 1, 2 and 4. In free alkali test, bar soap that qualified the requirements was formula 2. Moringa leaves ethanol extract bath bar soap in formula 1 to 5 has weakness antioxidant activity.

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