



Research Article

Formulation and Evaluation of Facial Wash containing Snow Mushroom (*Tremella fuciformis*) Extract

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ABSTRACT

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The aims of this study were to determine and evaluate the results of the physicochemical characteristic of snow mushroom (*Tremella fuciformis*) facial wash and to determine the acceptability of the snow mushroom (*Tremella fuciformis*) extract facial wash which has the best physicochemical characteristics. In this study, the extraction of snow mushroom (*Tremella fuciformis*) used UAE (Ultrasound-Assisted Extraction) and ethanol as a solvent with various concentrations, 96% (F1), 75% (F2), and 50% (F3). Furthermore, the extract will be formulated into a facial wash and evaluated for physicochemical characteristics. The parameters of physicochemical characteristics include organoleptic, pH, density, viscosity, spreadability, and foamability. The difference in the concentration of ethanol as menstruum of snow fungus (*Tremella fuciformis*) extraction did not significantly affect the organoleptic composition of facial wash, but it did affect the physicochemical characteristics of facial wash, where higher ethanol concentrations resulted in lower pH, higher density and viscosity, smaller spreadability, and foamability that meets specifications. The results of the evaluation of physicochemical characteristics showed that F1 had the best specifications and continued hedonic testing to determine the acceptability of snow mushroom (*Tremella fuciformis*) extract facial wash. The results of the hedonic test on F1 showed good acceptance regarding the parameters of appearance, foam, and effects after using facial wash; as well as sufficient acceptability related to the aroma and viscosity parameters.

1. INTRODUCTION

Mushrooms that are used as ingredients in pharmaceutical cosmetic products act as cosmeceuticals (treatment) and nutricosmetics (nutrition) (Wu *et al.*, 2016). Snow mushrooms (*Tremella fuciformis*), belonging to the order Tremellales and family Tremelaceae, contain polysaccharides consisting of a linear (1 → 3)-linked α -mannose backbone, most of which are β -xylose and β -glucuronic acid. Snow mushroom (*Tremella fuciformis*) polysaccharides were extracted optimally under alcohol compared to hot water solvents (An *et al.*, 2019).

When *T. fuciformis* polysaccharides are present on the surface of the skin, a film will be formed which can increase the level of water retention, so that the water content in the skin is maintained so that the skin does not lose its moisture (Lin and Tsai, 2019). *T. fuciformis* polysaccharides can also improve the skin barrier on the skin so that skin moisture is maintained (Ma *et al.*, 2021). This shows that snow fungus (*Tremella fuciformis*) has the potential as an effective moisturizing agent in cosmetic products, one of which is a facial wash.

In this study, the extraction of snow mushroom (*Tremella fuciformis*) using ethanol in various concentrations, ethanol 96% (F1), 75% (F2), and 50% (F3) using the Ultrasound-Assisted Extraction (UAE) method. The selection of the menstruum is based on the principle of "like dissolves like", where the appropriate solvent for extraction is a solvent with a polarity similar to the compound to be extracted. The UAE method was chosen because it can improve extraction efficiency. Ultrasonic waves will produce cavitation which can increase the contact time of the simplicia with the solvent and increase the permeability of the cell wall thereby accelerating the dissolution and diffusion of solutes (Nn, 2015).

The UAE snow mushroom (*Tremella fuciformis*) extract will be concentrated to obtain a thick extract, which will then be subjected to a Molisch test and pH test. The concentrated extract of snow mushroom (*Tremella fuciformis*) was then formulated into a facial wash and evaluated for physicochemical characteristics related to organoleptic parameters, pH, density, viscosity, spreadability, and foamability. It aims to determine the effect of the concentration of ethanol as an extraction mechanism for snow mushroom (*Tremella fuciformis*) on the physicochemical characteristics of facial wash preparations. The preparation of snow mushroom (*Tremella fuciformis*) facial wash with the best specifications was carried out by a hedonic test to determine its acceptability.

2. MATERIALS AND METHODS

Materials and Tools

The materials used in this study for extraction were snow mushroom (*Tremella fuciformis*), 96% ethanol (technical grade), and aqudest. The materials used in the formulation of facial wash were virgin coconut oil (VCO), KOH, stearic acid, CMC-Na, BHT, DMDM hydantoin, Sodium lauryl

sulfate, CAPB, Comperlan, Na-EDTA, propylene glycol, glycerin, and fragrance with pharmaceutical-grade specification.

The tools used in this study were gram scale, UAE (Power Sonic 405), pH meter (Schott Lab 850), a set of spreadability test equipment, cone and plate viscometer (Brookfield type cone and plate series AT 71362), a set of foambility test equipment, hot plate stirrer, and a set of glass tools.

Methods

Extraction

30 grams of dried snow mushroom (*Tremella fuciformis*) blended into powder, then transferred into three beakers labeled F1, F2, and F3. 100 mL of ethanol was put into the labeled glass beakers which F1: 96% ethanol; F2: 75% ethanol; F3: 50%. The F1, F2, and F3 beakers were extracted by the UAE method at a temperature of 50°C for 30 minutes. The extraction was replicated three times. The extracted filtrate is concentrated to obtain a thick extract that will be used for the formulation of facial soap preparations.

Evaluation of Extract (Molisch Test dan pH test)

Molisch test: 1 ml of thick extract was put into a test tube, then add 2 drops of Molisch reagent and shake gently, then add 0.5 ml of concentrated sulfuric acid. The Molisch test is said to be positive if a red-purple ring is formed.

pH test: dip the indicator in the extract sample, then compared it with the existing scale on the universal indicator to determine the pH value of the extract.

Formulation of Facial Wash

No.	Materials	(%)	For 150 gram	Function
1.	Snow Mushroom Extract (<i>Tremella fuciformis</i>)	5	7,5 g	API
2.	Stearic Acid	0,5	0,75 g	Emulgator
3.	Carboxymethylcellulose-Na	0,5	0,75 g	Gelling agent
4.	Butylated Hydroxytoluene	1	1,5 g	Antioxidant
5.	Kalium Hidroxyde	6,4	9,6 g	Basic base
6.	Virgin Coconut Oil	30	45 g	Fatty acid-base
7.	DMDM Hydantoin	0,2	0,3 g	Preservative
8.	Sodium Lauryl Sulfate	4	6 g	Foaming agent
9.	Cocamidopropyl Betaine	4	6 g	Surfactant
10.	Cocamide DEA	4	6 g	Surfactant
11.	Na-EDTA	0,1	0,15 g	Chelating agent

12.	Propylene Glycol	2	3 g	Humectant
13.	Glycerin	2	3 g	Humectant
14.	Fragrance	0,3	0,45 g	Perfume
15.	Aquadestillata	40	60 g	Solvent

The materials were mixed on the hotplate stirrer at 70-80°C with a speed of 400-500 RPM.

Evaluation of Facial Wash

a) Organoleptic

a. Organoleptic

The organoleptic test included appearance, smell, texture, and color. The appearance, texture, and color of the product were tested by direct visual observation. The smell of facial wash was tested by smelling the scent of the product.

b. pH

The pH test of the facial wash with snow mushroom (*Tremella fuciformis*) extract was carried out with a pH meter (Schott Lab 850).

c. Density

A calibrated beaker with a volume of 15 ml is weighed first (m_1), then 1-2 grams of facial wash is added and then weighed (m_2). In the measuring cup, 15 ml of distilled water was prepared, then the aquadest was poured slowly up to the calibration mark of the beaker glass, and recorded the remaining volume of distilled water in the measuring cup (V). Density is obtained by the following calculation.

$$\rho = \frac{(m_2 - m_1)}{V}$$

d. Viscosity

The viscosity test of the facial wash with snow mushroom (*Tremella fuciformis*) extract was carried out with a cone and plate viscometer (Brookfield type cone and plate series AT 71362).

e. Spreadability

0.5 grams of facial wash were pressed with a load of 50 grams to 250 grams for 1 minute, then the diameter of each additional load was recorded until it was constant.

f. Foamability

1 mL of the facial wash is dissolved in 10 mL of distilled water in a test tube. The facial wash solution was shaken for 20 seconds by inverting the test tube, then the height of the foam was measured

g. Hedonic Testing

This test was conducted using 20 male and female respondents aged 15-50 years. Before the hedonic test, respondents were asked to do an allergy test by applying the preparation to the skin behind the ear. The test was carried out by distributing the product to be tried by the respondents, then the respondents filled out a questionnaire containing the evaluation parameters, which included appearance, aroma, viscosity, foam, and the effect felt after using facial wash.

3. RESULTS AND DISCUSSIONS

*Snow Mushroom (*Tremella fuciformis*) Extract*

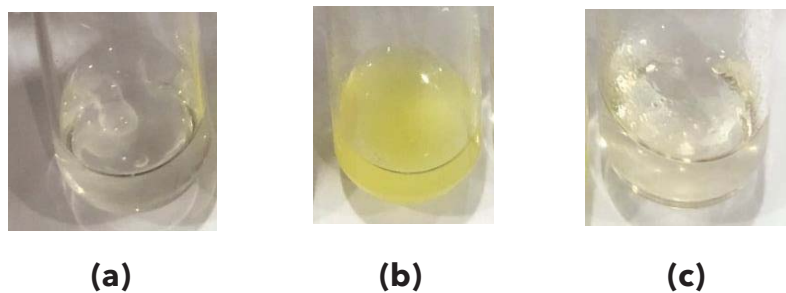


Figure 1. Snow Mushroom (*Tremella fuciformis*) Extract:
(a) Extract ethanol 96%; **(b)** Extract ethanol 75%; **(c)** Extract ethanol 50%

The color of the snow mushroom extract produced corresponds to the color of the snow mushroom extract which contains polysaccharides on the market, which is white or yellowish (SpecialChem. 2021). This proves that the extract obtained contains polysaccharides visually, then the Molisch test is carried out to ensure the polysaccharide content.

*Evaluation of Snow Mushroom (*Tremella fuciformis*) Extract*

a. Molisch Test

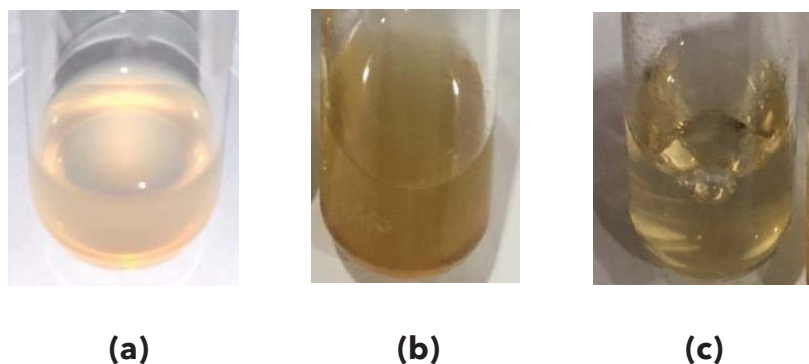


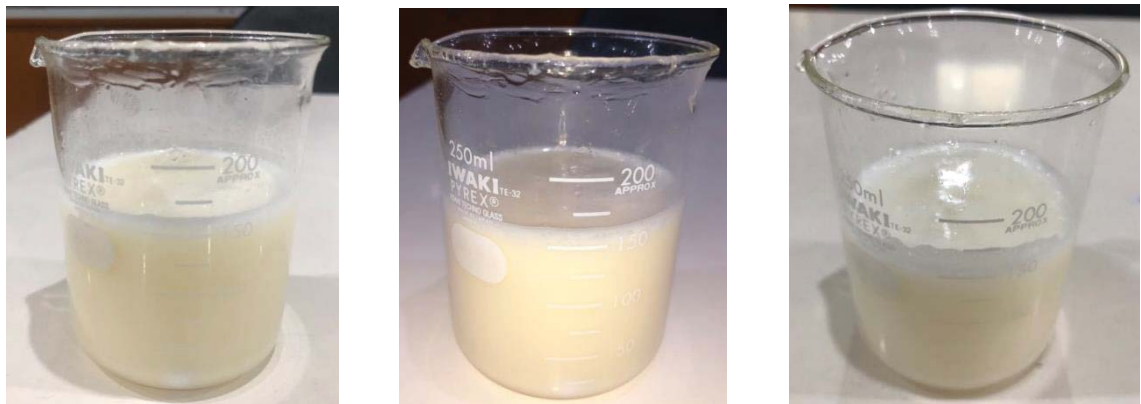
Figure 2. Molisch test of Snow Mushroom (*Tremella fuciformis*) Extract:
(a) Extract ethanol 96%; **(b)** Extract ethanol 75%; **(c)** Extract ethanol 50%

The Molisch test gives a positive reaction if a red-purple ring is formed. The more concentrated the red-purple color, the higher the carbohydrate content in the extract. Snow mushroom (*Tremella fuciformis*) extract in 96% ethanol showed a faint red ring, 75% ethanol extract formed a fainter red ring, and 50% ethanol extract formed a faint pink ring. The three extracts showed a positive reaction to the Molisch reagent, so it can be said that they contain carbohydrates (polysaccharides) even though the ring formed is red/pink in color. The faded color is because polysaccharides are carbohydrates that need to be hydrolyzed into monosaccharides to react with Molisch reagent. The faded color can also be due to the relatively low amount of carbohydrates in the extract. Judging from the color density produced, 96% ethanol extract has the highest polysaccharide content compared to 75% and 50% ethanol extract (96% > 75% > 50%). This is also because ethanol solvent has optimal polarity in extracting polysaccharides, as has been found in previous studies where alcohol is more optimal for extracting snow fungus (*Tremella fuciformis*) than hot water (An et al., 2019).

b. pH Test

Snow mushroom (*Tremella fuciformis*) extract in 96% ethanol showed pH 3,67; 75% ethanol extract showed pH 4,21, and 50% ethanol extract showed pH 6,12. This pH is quite acidic because snow mushroom extract contains polysaccharides whose main constituents are β -xylose and β -glucuronic acid (Lin and Tsai, 2019). pH valued measured with pH meter Schott instruments. β -glucuronic acid is a class of carboxylic acid compounds that can provide an acidic atmosphere to the extract. The snow mushroom (*Tremella fuciformis*) extract products on the market also have a pH that tends to be acidic, namely pH 5.5 - 7.5 (SpecialChem).

Snow Mushroom (*Tremella fuciformis*) Extract Facial Wash



(a) (b) (c)
Figure 3. Snow Mushroom (*Tremella fuciformis*) Extract Facial Wash:

(a) Extract ethanol 96%; (b) Extract ethanol 75%; (c) Extract ethanol 50%

Evaluation Snow Mushroom (Tremella fuciformis) Extract Facial Wash

No.	Parameters	Spesification	F1	F2	F3
1.	Organoleptic :				
	Form	Liquid	Liquid	Liquid	Liquid
	Color	White/white Cream	White	White Cream	White
	Aroma	Perfume	Perfume	Perfume	Perfume
	Texture	Homogeneous	Homogeneous	Homogeneous	Homogeneous
2.	pH	10,00 - 11,00	10,67	10,91	11,07
3.	Density	0,50 - 0,70 g/cm ³	0,67 g/cm ³	0,60 g/cm ³	0,58 g/cm ³
4.	Viscosity	150,0 - 1000,0 cps	975 cps	231,1 cps	184,9 cps
5.	Spreadability	80-100%	90%	80%	100%
6.	Foamability	7,5 - 14,0 cm	7,6-9,8 cm	10,2-13,6 cm	10,1-13,5 cm

Organoleptic testing was carried out to determine the visual appearance of the products. The three formulas (F1, F2, and F3) showed organoleptic observations that were following the specifications of the facial wash, which is liquid form, white or cream-white in color, smelled typical of perfume, and was homogeneous.

The three preparations produced pH in accordance with the specifications (pH 10.0-11.0). F3 produces a pH that slightly exceeds the specifications. The pH adjustment at F3 could not be carried out because the addition of acid made the facial wash form in two phases and unstable. Analysis of the results of observing differences in pH characteristics of the facial wash preparations of snow mushroom (*Tremella fuciformis*) extracts in formulas I, II, and III using one-way ANOVA show a probability value or P-Value of 0.000, which is a P-value <0.05, so it can be concluded there was a significant difference between pH and various concentrations of ethanol used for the extraction of snow mushroom (*Tremella fuciformis*) extract in the formulation of facial soap preparations. The concentration of ethanol as an extractor for the snow mushroom (*Tremella fuciformis*) indicates that the higher the concentration, the lower the pH of the facial soap preparation, where in this study 96% ethanol extract had the lowest pH compared to 75% and 50% ethanol extract (pH F1<F2<F3).

F1, F2, and F3 produced density in accordance with the specifications for facial wash preparations with snow mushroom (*Tremella fuciformis*) extract, which were 0.50-0.70 g/cm³. The concentration of ethanol as an extractor for the snow mushroom (*Tremella fuciformis*) indicates that the higher the concentration of ethanol as a menstruum, where in this study 96% ethanol extract

had the highest density compared to 75% and 50% ethanol extracts (density $F1 > F2 > F3$). This was because the snow mushroom (*Tremella fuciformis*) was optimally extracted at 96% ethanol, the amount of polysaccharide extracted was more so that the density produced would be greater.

The three preparations produced a viscosity in accordance with the specifications (150.0-1000.0 cps). Viscosity is proportional to the density, and in this study, F1 has the highest viscosity and density ($F1 > F2 > F3$). Analysis of the observed differences in viscosity characteristics of the facial soap preparations of snow mushroom (*Tremella fuciformis*) extracts in formulas I, II, and III using one-way ANOVA showed a probability value or P-Value of 0.000, which $P\text{-value} < 0.05$, so it can be concluded there was a significant difference between the viscosity and various concentrations of ethanol used for the extraction of snow mushroom (*Tremella fuciformis*) extract in the formulation of facial wash preparations. The concentration of ethanol as an extraction method for snow mushroom (*Tremella fuciformis*) indicates that the higher the concentration, the greater the viscosity of the facial wash (Viscosity $F1 > F2 > F3$).

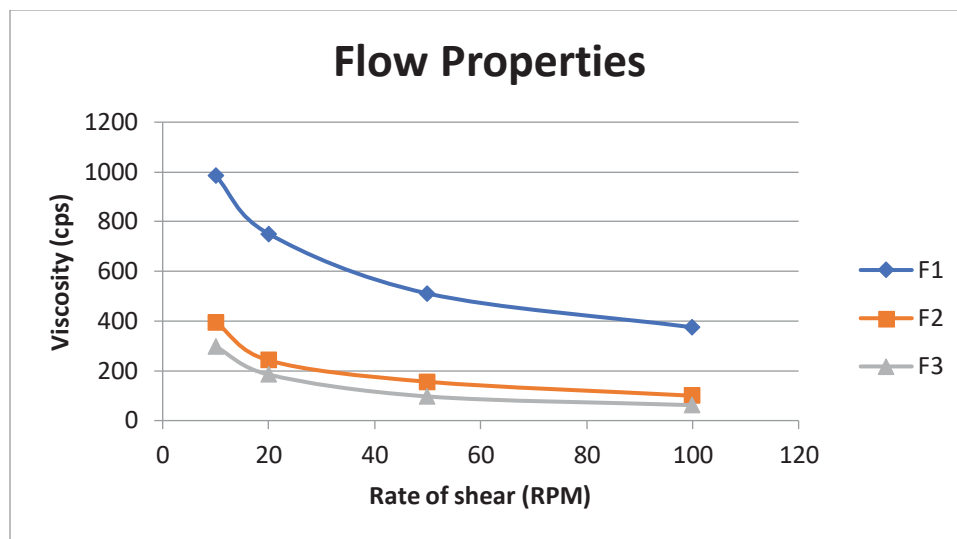


Figure 4. Graph of flow properties of facial wash with snow mushroom (*Tremella fuciformis*) extract

Viscosity measurement in this study uses a cone and plate viscometer which is included in multi-instrument so that it can test the viscosity of the sample at different rates of share (RPM) to determine its flow properties. **Figure 4** shows that the three preparations have pseudoplastic flow properties, where the viscosity will be lower when the shear stress increases. Pseudoplastic flow properties in the facial wash will facilitate the take out of the soap from primary containers such as tubes.

The three preparations showed the results of the spreadability measurement in accordance with the specifications of the facial wash preparations with snow mushroom (*Tremella fuciformis*) extract, which was 7.5-14.0 cm. Viscosity affects dispersion inversely, where the higher the

viscosity, the smaller the spreadability obtained (Eugresya, Avanti, and Uly, 2017). F1 has the highest viscosity, so it has the smallest spreadability compared to F2 and F3.

F1, F2, and F3 produced foamability in accordance with the specifications (80-100%). Analysis of the results of observing differences in foam height characteristics from facial soap preparations of snow mushroom (*Tremella fuciformis*) extracts in formulas I, II, and III using one-way ANOVA show a probability value or P-Value of 0.037, which is a P-value < 0.05, so that it can be concluded that there is a significant difference between foamability and various concentrations of ethanol used for the extraction of snow mushroom (*Tremella fuciformis*) extract in the formulation of facial wash preparations.

Hedonic Testing

Hedonic test was carried out on the snow mushroom (*Tremella fuciformis*) extract facial wash in 96% ethanol (F1). It was chosen as the hedonic test sample because the evaluation results met the specifications for the desired facial wash, which was in the form of a liquid soap that was homogeneous, white in color, and had a distinctive perfume smell. F1 has good viscosity, spreadability, and foam according to specifications, as well as the lowest pH compared to F2 and F3, where the pH is 10, and the closest approach to soap preparations is generally around pH 9-10 (Draelos, 2017). The selection of snow mushroom (*Tremella fuciformis*) extract facial wash in 96% ethanol was also based on the highest polysaccharide content compared to 75% and 50% ethanol extract.

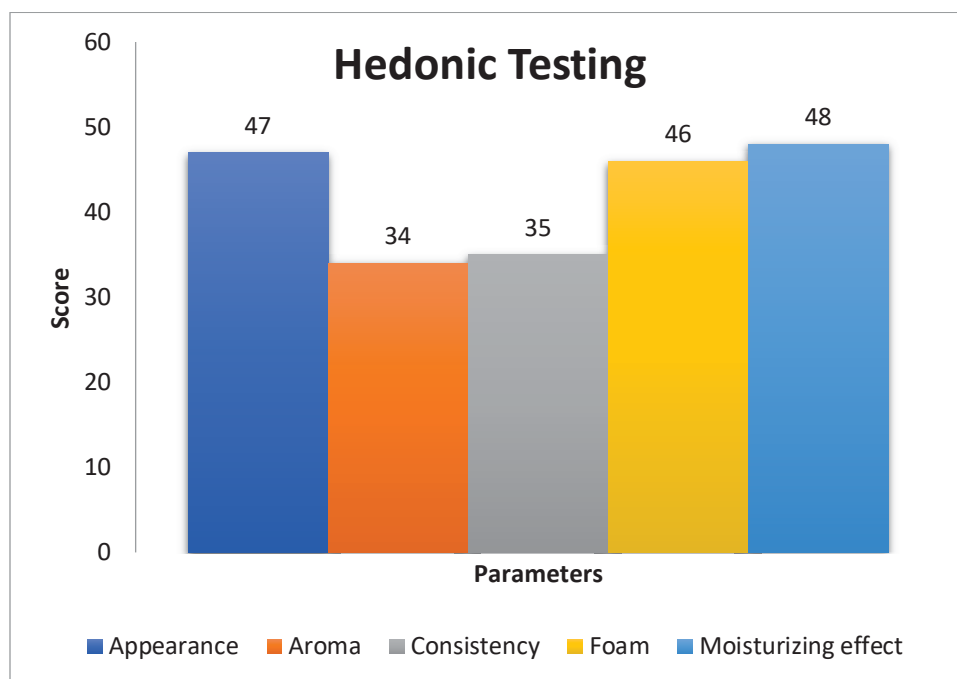


Figure 5. Snow Mushroom

The hedonic test scoring scale is a score of 1 (enough), 2 (like), and 3 (very like), so the maximum total score that can be obtained for each parameter is 60. In Figure 5 it is known that the score for each parameter is the appearance (47), aroma (34), consistency (35), foam (46), and the effect felt after using facial soap/moisturizing effect (48). On average, the aroma and consistency of facial soaps get a sufficient rating, so changes need to be made to increase its acceptability by adding perfume to increase the aroma of the product, as well as adding a thickening agent to increase the viscosity of facial wash. Appearance, foam, and the effect felt after using facial soap/moisturizing effect on average received a favorable rating, which indicates a good acceptance of these parameters.

4. CONCLUSIONS

The difference in the concentration of ethanol as an extraction menstruum for snow mushroom (*Tremella fuciformis*) has an effect on the physicochemical characteristics of facial wash preparations, where higher ethanol concentrations result in lower pH, higher density and viscosity, smaller spreadability, higher foamability, as well as organoleptic which does not affect the difference in ethanol concentration as an extraction menstruum. F1 showed as the best formula of snow mushroom (*Tremella fuciformis*) facial wash, that also had good acceptance regarding the parameters of appearance, foam, and effects after using facial wash (moisturizing effect); as well as sufficient acceptability related to aroma and viscosity parameters.

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