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Formulation of Scrub Preparations With Variations in the Concentration of Seagrass Leaf Ethanol Extract (Enhalus *acoroides)*

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Abstract

Seagrass leaves (Enhalus acoroides) are known to contain antioxidants that can protect the skin from free radicals and keep the skin's pH stable. Group compounds found in seagrass leaf components are fernolics, tannins, flavonoids, and glycosides. To facilitate the application of seagrass leaf extract, it is necessary to formulate one of them, namely scrub preparations. Seagrass leaf ethanol extract formulation uses variations in concentrations of F1 20%, F2 30% and F3 40%. The purpose of the study was to determine physical properties tests, irritation tests and moisture tests on seagrass leaf ethanol extract scrub preparations. Seagrass leaf extract was extracted by remaceration method using 70% ethanol then formulated into scrub preparations. Scrub is a liquid or semisolid preparation in the form of an emulsion. The results of the physical properties test of scrub preparations showed semisolid preparations of green to dark green color, had a characteristic seagrass odor and were homogeneous because they did not show the presence of coarse grains. F1 viscosity results 39,260, F2 results 39,278, F3 results 39,385, dispersion tests obtained F1 7.93, F2 results 8 and F3 results 7.73, then F1 adhesion test results 5.63, F2 results 5.75, F3 results 7.14 and F1 pH test results of 5.7, F2 results 5.6 and F3 5.6. In the irritation test results, the three formulas showed no irritation and the moisture test of the three formulas was less moist. It can be concluded that the results of the physical properties test of viscosity, pH, adhesion and dispersion tests are in accordance with the range required, the irritation test in the three formulas does not occur irritation and in the moisture test the results obtained are moist.

Keywords: Scrub, seagrass, Leaf, Extract, Concentration varian

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INTRODUCTION

Regular skin care is the first step to improving and maintaining the skin's natural beauty. One way to treat dry skin naturally is to use traditional exfoliating ingredients. Traditional body scrubs are cosmetic preparations made from fresh natural ingredients or dried plants or fruits (Fauzi *et al.*, 2012).

Secondary treatments can prevent skin aging by using cosmetic products that contain antioxiandts such as polyphenols. Sunlight often irritates the skin because UV A and UV B rays emitted by the sun can stimulate the formation of reactive oxygen species. This causes oxidative stress to appear in the skin when the

amount of ROS (reactive oxygen species) exceeds the ability of skin cells to neutralize their antioxiandts (Poljsak, 2012).

Seagrass leaves (*Enhalus acoroides*) is a natural ingredient that contains antioxidants. Seagrass leaves contain phenolic compounds, tannins, flavonoids and glycosides (Dewi and Mirna, 2012). Research conducted says that seagrass leaf ethanol extract has a very strong antioxidant activity, with IC value$_{50}$ 38 ug/m (Sami *et al.,* 2020)

Body scrub made from seagrass leaf extract (*Enhalus acoroides*). An extract is a concentrated preparation obtained by extracting the active substance from vegetable or animal plants using an appropriate solvent, followed by evaporation of all solvents, the remaining mass is treated in such a way that it meets predetermined standards. Extraction is a method of separating a mixture of several substances into separate components (Darmayanti and Fitriana, 2011).

Until now, no research has been conducted on the manufacture of products *body scrub* from seagrass leaf ethanol extract (*Enhalus acoroides*). Lulur is a semi-solid preparation derived from natural ingredients in the form of plants, which is used to remove dead skin cells, clean dirt and open clogged pores. It is intended to achieve healthier, glowing skin, and have a lighter skin tone (Maryam, 2017).

RESEARCH METHOD

Laboratory experimental method where body scrub is made from seagrass leaf extract (*Enhalus acoroides) using the matching rema method*for 5 days with 70% ethanol. The results of seagrass leaf extract *(Enhalus acoroides)* will be made into *body scrub preparations* with varying concentrations of seagrass leaf extract *(Enhalus acoroides*) of 20%, 30% and 40%. Physical stability tests are carried out on preparations including organoleptic tests, homogeneity tests, pH tests, viscosity tests, dispersion tests, adhesion tests, irritation tests and moisture tests.

RESULTS AND DISCUSSION

1. Seagrass leaf plant determination

The plants used in this study were recognized or identified first. Seagrass leaves (*Enhalus acoroides*) are obtained from Batu Gong Village, Lalunggasumeeto District. Konawe County. Seagrass leaf plants are taken from the coastal waters of Batu Gong, Southeast Sulawesi. Seagrass leaves (*Enhalus acoroides*) were determined at the Biological Laboratory of Universitas Ahmad Dahlan. The identification process aims to ascertain the type of plant used in the study, with the aim of preventing errors in sampling. Based on the results obtained, family: Hydrocharitaceae, genus: Enhalus, Species: Enhalus acoroides showed that the plants used in this study actually came from the seagrass leaf type Enhalus acoroides.

2. Results of Seagrass Leaf Extract (*Enhalus acoroides*)

In this study, extracts were made using simplisia from seagrass leaves (*Enhalus acoroides*) A total of 1.400 grams of simplisia powder was carried out by remaceration process with 70% ethanol for 5 days after which the solvent evaporation process from the remaceration results was carried out

using a rotary vacuum evaporator at a temperature of 56^0C until the ethanol solvent is reduced (±1.5 hours). The extract is then processed *waterbath* at a temperature of 70°C. Until the results of thick seagrass leaf extract as much as 102.66 grams were obtained with a calculated yield value of 7.33%, which results were not in accordance with the requirements for a good seagrass leaf ration value, which was not less than 10% (Fitriyah, 2013).

The results in this study that are not in accordance with the requirements of a good arrangement can be influenced by the effectiveness of the extraction process. According to there are several factors that can affect the extraction yield such as time, temperature, stirring and solvent type (Febrina *et al.*, 2015). In addition, the sample size can also affect the number of results obtained where the smaller the sample surface area, the less the extract results, while if the sample size of seagrass leaves (*Enhalus acoroides*) the greater the chance, the wider the contact between the solvent and the sample which increases the interaction with the solvent resulting in more viscous extracts (Sineke, 2016).

3. Results of Making Seagrass Leaf Extract Scrub (*Enhalus acoroides*)

In this study, scrub preparations were produced with varying concentrations of seagrass leaf extract (*Enhalus acoroides*), which are 20%, 30% and 40%. Honey was chosen because it has natural moisturizing properties. The reason for choosing honey as a natural ingredient is because of its ability to make the skin moisturized, increase skin elasticity and firmness (Rahayu, 2013).

The next ingredient used is rice flour, which acts as a natural scrubbing agent because it has various benefits for skin health, because rice can help improve skin elasticity. The type of preservative used in this study was a combination of methyl paraben and propyl paraben preservatives. The selection of a combination of preservatives is carried out due to the growth of microorganisms can live in the water and oil phases. The use of a single preservative is less effective against the coefficient between oil and water, i.e. both cannot be distributed (Musdalipah and Reymon, 2018).

One of the ingredients as an emulsifier that is widely used in scrub preparations type (O/W) is stearic acid and triethanolamine. Stearic acid functions as an emulgator which in making scrub preparations if reacted with potassium hydroxide or triethanolamine bases used can neutralize scrub preparations. Triethanolamine material will form a very stable emulsion when used in conjunction with free fatty acids such as strearic acid (Saryanti *et al*., 2019). Furthermore, the distilled water material in this study was used as a solvent to dissolve the ingredients in making scrub preparations to be homogeneous.

4. Ethanol Free Test Results for Seagrass Leaf Extract (*Enhalus acoroides*)

Ethanol-free tests are carried out to ensure that the extracts obtained or to be sampled in the testing of this scrub preparation are ethanol-free. Seagrass leaf extract that has been positive for ethanol-free can be used because the extract is pure without contamination and to avoid the appearance of false positives in the next sample treatment (Samsumaharto and Erlina, 2014).

Table 1. Ethanol-free test results of seagrass leaf extract

Identification	Result	Standard
Seagrass leaf extract 2 ml + concentrated H2SO4 2 drops	It is green in color, has the characteristic smell of seagrass leaves and does not have the smell of esters	The smell of esters in the extract disappears
Seagrass leaf extract 2 ml + concentrated H2SO4 2 drops +acetic acid	Green in color, the smell of esters disappears	

The results of the ethanol-free test in this study obtained the results of a typical green liquid derived from seagrass leaves and there was no ester smell typical of ethanol so that it was tested positive for ethanol-free (Samsumaharto and Erlina, 2014).

5. Physical Properties Test Results of Seagrass Leaf Extract Scrub (*Enhalus acoroides*)

a. Organoleptic Test

Organoleptic tests are carried out by looking at phase changes or separations, appearance of odor or not, dosage form and color changes before and after storage. Based on the results of observations on the three scrub formulas, it was found that the scrub was semi-solid which became thicker with increasing extract concentration and the scrub did not clump and had a uniform texture in each test.

The colors produced in the three formulations vary. The color in formula 1 is light green, in formula 2 it is dark green and in formula 3 it is dark green. The green color of the scrub comes from seagrass leaf extract which is typical of light green to dark green (Juwita *et al*., 2013).

The scrub preparation has the most intense color is formula 3, this is because formula 3 contains the most amount of seagrass leaf extract, which is 40 grams compared to the other two formulas that contain less seagrass leaf extract, namely, 30 grams in formula 2 and 20 grams in formula 1.

The smell produced in the three scrub formulas has a distinctive aroma of seagrass extract which is not too pungent but the smell of the scrub will be more intense along with the increase of seagrass leaf extract in each formula in the scrub preparation (Juwita *et al*., 2013).

It is known that the scrub results in the three test formulas show a slight difference in the intensity of viscosity, color and odor that is different in each formula, this is influenced by the amount of extract concentration used in each formula in scrub preparations where the scrub will be thicker, darker in color and smell more intense if the concentration of seagrass leaves in the scrub preparation is more (Kusuma *et al*., 2021).

b. Homogeneity Test

The homogeneity test aims to determine whether the seagrass leaf extract scrub preparation is mixed evenly during preparation and there are no viscous particles in the scrub preparation. If the scrub preparation is not homogeneous, it can be concluded that it is unstable and there is less

dispersed solid material that will affect the time of use (Sustainable *et al*., 2021).

The homogeneity test results after each repetition was carried out three times for each test formula showed that the preparation that had been made could be declared homogeneous, because there were no grains When the preparation is tested on transparent glass because the ingredients used melt perfectly during the process of making scrub preparations. If the scrub is homogeneous, then all parts of the skin have an equal opportunity to benefit from the substances contained in the preparation (Mektildis, 2018).

c. pH Test

The purpose of pH testing is to determine the acidity level of body scrub preparations so that they match the physiological pH. The pH range requirement for a good topical product is 4.5-6.5. The acidity level is too acidic and too alkaline which will cause irritation to the skin. In addition, the pH of the scrub preparation should be within the pH range of the skin to prevent skin irritation (Nasution *et al.*, 2022).

Table 2. pH test results

Formula	x̄± SD	*Standard*
1	5.7 ± 0.10	
2	5.6 ± 0.15	4.5 – 6.5
3	5.5 ± 0.15	

The test results showed that there was a difference in the pH of each preparation which was influenced by the large concentration of seagrass leaf extract used, which the more it produced a more acidic pH. Increasing the concentration of seagrass leaf extract will reduce the pH value of the preparation so that the pH of formula 2 will decrease to formula 3 which has a lower pH value because formula 3 has the highest extract concentration so that the pH becomes low (Leny *et al.,* 2020).

Data from the pH test results of seagrass leaf ethanol extract scrub preparations were obtained from the analysis of normality test and homogeneity test. The normality test results show that the data is normally distributed with the significance value resulting from the test result >0.05. Homogeneity shows that the data is homogeneous with a significance value of >0.05. Because the data is normally distributed and homogeneous, it is continued with the ANOVA oneway test. In the test, the result p=0.842>0.05 was obtained which stated that there was no significant difference in pH parameters in the scrub formula with the concentration of seagrass leaf ethanol extract.

d. Viscosity Test

Viscosity testing aims to determine the viscosity of a preparation, the higher the viscosity value, the thicker the preparation and vice versa, the lower the viscosity value, the more liquid the preparation (Iskandar *et al.*, 2021). The viscosity measurement results of seagrass leaf ethanol extract scrub preparations with three replications have viscosity values that are in accordance with the dosage quality standards set by SNI 16-4399-1996, namely 2.000-50.000 cps (Iskandar *et al.*, 2021).

Table 3. Viscosity test results

Formula	x̄± SD	*Standard*
1	39.260 ± 0.006	
2	39.278± 0. 030	2.000 – 50.000
3	39.385 ± 0.030	

Viscosity test results Seagrass leaf ethanol extract scrub preparation It can be known that From Formula 1-3 shows the result that the viscosity value continues to increase, which in Formula 3 has the largest viscosity value compared to the other two scrub formulas. The increase in the viscosity value of formula 3 occurs along with an increase in the concentration of the extract Seagrass leaves The higher the concentration of extracts in formula 3 can increase the amount of polymer that undergoes *Cross link* which will form the basis Seagrass leaf ethanol extract scrub preparation thus increasing viscosity (Judge *et al.*, 2020).

The data obtained from the viscosity test of seagrass leaf ethanol extract scrub preparations were carried out normality test analysis (*Shapiro-Wilk*) and homogeneity test. The normality test results show that the data is normally distributed with a significance value of >0.05. The homogeneity test results showed that the data were homogeneous with a significance value of >0.05. Because the data is normally distributed and homogeneous, it is continued with the one-way ANOVA test p= 0.004<0.05 which states that variations in the concentration of seagrass leaf ethanol extract in scrub preparations have significant differences in viscosity parameters. To see a significant difference between the formulas, we proceed with the *post hoc Tukey* HSD test. The result is formula 1 to formula 2 significant with a value of 0.005 and vice versa, while formula 2 to formula 3 is significant with a value of 0.01.

e. Adhesion Test

The purpose of the adhesion test is to determine the time required for the preparation of the scrub to be attached to the skin (Prabanandi and Suherman, 2018). Adhesion is the ability of the preparation to stick to the skin for a long time when used. The greater the adhesion value of the preparation, the better because the penetration time of the scrub preparation into the skin, the more optimal.

Table 4. Adhesion test results

Formula	x̄± SD	*Standard*
1	5.63 ± 0.18	
2	5.75 ± 0.32	>4 seconds
3	7.14± 0.58	

The results of the adhesion test that can be seen in formula 3 with a concentration of seagrass leaf ethanol extract of 40% have a higher adhesion value compared to formula 1 and formula 2, so according to previous studies, the higher the concentration of the extract used, the denser the preparation so that the adhesion produced will last longer (Mectyldis, 2018). Stickiness of seagrass leaf ethanol extract scrub with triple replication It can be seen that the adhesion value of scrub

preparations shows a greater value along with the increase in the concentration of seagrass leaf extract because with the increasing extract, the scrub becomes thicker so that the adhesion value becomes even greater. So it can be known that the results of the adhesion test are proportional to the viscosity value and inversely proportional to the dispersion value (Sopianti and Saiful, 2022).

Data from the adhesion test results of seagrass leaf ethanol extract scrub preparations obtained were subjected to normality test analysis (*Shapiro-Wilk*) and homogeneity test. The normality test results show that the data is normally distributed with a significance value of >0.05. The homogeneity test results showed that the data were homogeneous with a significance value of >0.05. Because the data was distributed normally and homogeneously, it was continued with the one-way ANOVA test with the value obtained p=0.007<0.05 which showed that there were differences in variations in the concentration of seagrass leaf ethanol extract in body scrub preparations that had a significant effect on adhesion parameters. To see the significant differences between formulas, we proceed with the *post hoc Tukey* HSD test. The result is formula 1 against formula 3 significant with a value obtained of 0.009, in formula 2 against formula 3 significant with a value obtained of 0.013.

f. Dispersion Test

Seagrass leaf ethanol extract formula distribution testing aims to determine the distribution of preparations on the skin when applied to the skin so that its distribution can be more optimal (Irma *et al.*, 2023). Dispersion power test of seagrass leaf ethanol extract scrub formulation using direct dispersion test equipment. Dispersion power test of seagrass leaf ethanol extract scrub formulation using direct dispersion test equipment (Judge *et al.*, 2020).

Table 5. Dispersion test results

Formula	$\bar{x}$± SD	*standard*
1	7.93 ± 0.330	
2	8.00± 0.100	5 – 7 cm
3	7.73 ± 0.120	

The results of the dispersion power test of seagrass leaf ethanol extract scrubs in the three test formulas decreased the dispersion value, namely from formula 1 by 7.93 cm decreased to 7.73 cm in formula 3. Based on these results, it can be seen that the dispersion value of scrub preparations shows a smaller value with increasing concentrations of seagrass leaf extract because with the thicker a preparation, the smaller the dispersion value. So it can be seen that the results of the dispersion test are inversely proportional to the viscosity value and adhesion (Sopianti and Saiful, 2022).

Data on the dispersion test of seagrass leaf ethanol extract scrub preparations were obtained from the analysis of the normality test (*Shapiro-Wilk*) and homogeneity test. Test The normality test results show that the data is normally distributed with a significance value of >0.05.

The homogeneity test results showed that the data were homogeneous with a significance value of >0.05. The results showed that the test data was normally distributed and homogeneous, so it was continued with the oneway ANOVA test. In ANOVA's one-way test, p=0.347>0.05 showed that there was no significant difference in the concentration of seagrass leaf ethanol extract in scrub preparations on dispersion power parameters.

6. Seagrass Leaf Extract Scrub Irritation Test Results (*Enhalus acoroides*)

The purpose of the irritation test is to find out whether the preparation carried out can cause irritation (Iskandar *et al.*, 2021). Preparation of irritation tests was carried out on 3 Albino rabbits (*Oryctolagus cuniculus*), consists of 2 female rabbits and 1 male rabbit. The choice of sex in male and female rabbits is due to the appearance of acute irritation of the skin, which can occur in both males and females (Judge *et al.*, 2018). Observations are made at 1, 24, 48 and 72 hours so that we can know there is a possibility of delayed irritant reactions on the skin (BPOM, 2020).

Table 6. Irritation test results

Formula	Test Results	Information	BPOM Standard,2022	
			Score	Information
0	0	No irritation	0.0 – 0.4	Very mild irritation
1	0	No irritation	0.0 – 0.4	Very mild irritation
2	0	No irritation	0.0 – 0.4	Very mild irritation
3	0	No irritation	0.0 – 0.4	Very mild irritation

Irritation test results Seagrass leaf ethanol extract scrub preparation Shows that all tested formulas are safe to use because they do not cause irritation to rabbit skin, do not experience erythema or edema reactions, this is because the active ingredients used in the formula are natural ingredients that are proven safe and additives that are proven to be safe, inert, non-irritating and non-toxic. This is in line with research from arabica coffee bean extract which does not cause irritation because the ingredients used are proven safe (Retty *et al*., 2021). In other studies stated that the addition of seagrass extract does not have an irritating effect if it comes into contact with the skin because seagrass is an abundant source of vegetable life and contains antioxidants (*Enhalus acoroides*) (Arif *et al.*, 2021).

7. Seagrass Leaf Extract Scrub Moisture Test Results (*Enhalus acoroides*)

The purpose of the moisture test is to look at the moisture level of the seagrass leaf ethanol extract scrub preparation when applied to the skin (Ginting *et al.*, 2023). The result of measuring skin moisture before use until the 5th day after using the preparation. Before the scrub preparation was applied, the moisture of the skin on the backs of the volunteers' hands was first measured using tool *Skin Moisture Analyzer* (Rohs®) (Ratih *et al*., 2019).

Table 7. Moisture test results

Browseer	Humidity/Moisture (%)						Humidity Levels (%)	Standard
	Before Treatment	After Using Scrub Cream Preparations						
		Day 1	Day 2	Day 3	Day 4	Day 5		
A	39	41	45	42	48	53	53	
B	41	41	43	45	47	58	58	
C	36	36	39	38	42	53	53	
D	32	32	37	39	43	55	55	
E	42	43	48	50	55	59	40,5	
F	38	38	39	43	48	48	55	
x̄±SD	38±3.6	38±3.9	41±3.9	43±4.4	47±4.6	47±4.6	56.8±12.8	
A	39	39	43	48	55	59	63,8	40%-60% (humid) up to >60% (very humid)
B	41	46	48	55	48	60	46	
C	36	36	40	45	49	58	61,1	
D	32	32	38	43	58	57	78,9	
E	42	44	48	55	55	60	42,9	
F	38	38	40	45	54	57	50	
x̄±SD	38±3.9	39±5,2	43±5,2	49±5,3	54±4.4	59±1.4	59±13.2	
A	39	39	44	44	48	50	28	
B	41	41	43	48	50	55	34,1	
C	36	36	39	41	47	58	61,1	
D	32	32	39	45	48	57	78,1	
E	42	42	45	49	53	56	36,6	
F	38	38	39	45	48	53	47,6	
x̄±SD	38±3.4	38±3.6	41±2.6	45±2.9	49±2,2	55±2.9	47.6±18.9	

From the test data, it can be seen that before using seagrass leaf ethanol extract scrub, all volunteer groups had humidity levels ranging from 32% - 42% with the average moisture value of all penalists in each formula was 38% which showed that the skin of the penalis' back was included in the less moist category because the moisture value was <40%. After the 5th day of use, all volunteer groups had humidity levels ranging from 48% - 60%. This showed that after using seagrass leaf ethanol extract scrub preparations, the skin on the back of the panelists' hands became moist. According to previous studies, the skin moisture scale of 40%-60% belongs to the category of moist skin (Ratih *et al*., 2019).

The results of the water content chart show that formula 2 with a seagrass leaf ethanol extract concentration of 30% has the largest percentage of water content of 57% compared to formula 3 with a seagrass leaf ethanol extract concentration of 40% with a percentage of water content of 47.6% while formula 1 with a seagrass leaf ethanol extract concentration of 20% has the smallest percentage of water content, i.e. 47%. The compounds contained in seagrass leaves provide moisture to the skin. Another research study also stated that seagrass leaf extract gel preparations were able to increase dry category skin moisture to normal category skin with a seagrass extract concentration of 2% (Arif *et al.*, 2021).

CONCLUSION

The results of testing on the physical properties of scrub preparations showed that the preparation was a semi-solid cream that was green to dark green, had a distinctive and homogeneous seagrass odor because it did not show the presence of coarse grains. Scrubs in formulas 1-3 have an average pH value of 5 which is acidic and viscosity values between 39.278-39.385 cPs which can stick for more than 4 seconds with a dispersion between 7.73-8.00 cm. The scrub

irritation test on the three albino rabbits did not cause irritation and the moisture test on the three seagrass leaf ethanol extract scrub formulas had a moisture value of 47%-59% which was categorized as moist to very moist.

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