Formulation and characterization of Methanol Extract Onchidiid Slug (*OnchidiumTyphae*): Evaluation of antioxidant Cream

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Abstract: The Onchidiid slug (*Onchidiumtyphae*) is Mollusca phylum that does not have a shell; they have antioxidant properties and taurine and can function as anticholesterol, antibacterial, and antioxidant. This study aimed to formulate, characterize, and evaluate the antioxidant activity. The formula's optimization is done using the Simplex Lattice Design method. The cream formulation formula was made by comparing the concentrations of triethanolamine and stearic acid, namely Formula 1 (2%:18%), Formula 2 (3.5%:16.5%), Formula 3 (2%:18%), Formula 4 (2.5%:17.5%), Formula 5 (4%:16%), Formula 6 (3%:17%), Formula 7 (3%:17%), and Formula 8 (4%:16%). The cream that had been formed was then tested for physical properties and stability at room temperature on the first day and 28th days. The cream formula that met the requirements for the best physical properties and stability test for 28 days was tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method (DPPH). The results showed that the combination of stearic acid and triethanolamine affected the physical properties of the preparation, namely, an increase in the concentration of stearic acid caused a decrease in dispersion. The best formula found in the prediction results is a combination of 16.16% stearic acid and 3.84% TEA with an adhesion value of 24.576 seconds, a spreadability of 6.546 cm2, and a pH of 5.25. The antioxidant activity assay showed that the IC_{50} value is 54.584 ppm. The result can be concluded that the Onchidiid slug cream formula has the potential as an antioxidant.

Keywords: ***Onchidiumtyphae*, antioxidant, formula cream, onchidiid slug extract ethanolic**

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INTRODUCTION

The aging process or aging is a biological reaction that occurs naturally and occurs in all living things, including the aging process of all body organs such as the heart, lungs, brain, kidneys, and skin[1–4]. The aging process is divided into two, namely, the Intrinsic Process and the Extrinsic Process. This process can occur due to time (internal factors) and the influence of sunlight, pollution, or unbalanced nutrition (internal factors)[5,6]. One of the aging processes is caused by the photo-oxidation process, which includes the release of Reactive Oxygen Species (ROS) by chromophores that absorb ultraviolet light. An increase in free radicals that exceeds typical causes a decrease in antioxidants that neutralize reactive oxygen species (ROS). Antioxidants are substances that, at small concentrations, can significantly inhibit or prevent oxidation that can cause damage to lipids, cell structure, and DNA[7].

In previous research, the antioxidant activity of the ethanolic extract of sea slug(*Discodoris. sp*) had antioxidant activity using the DPPH free radical reduction method, with the enormous antioxidant potential of ethanol extract with an IC_{50} of 441.12 ppm[8–10]. Another study concluded that the antioxidant activity (IC_{50}) in meat extracted with ethanol was 150.92 ppm and the compound suspected to be an antioxidant was squalene[8,11]. The physicochemical characteristics of the extract of the sea slug species have a brown color with a thick consistency. However, the use of sea snails as a source of antioxidants for the skin is still rarely used by the public, so it is necessary to develop the extract into a topical dosage form. One of the topical preparations is in the form of a cream, where the use of cream as a cosmetic preparation can be used as a skin treatment to overcome the effects that can be caused by free radicals formed from oxidative metabolism and prevent premature aging[12,13]. Antioxidant cream preparations can be formulated with cream ingredients, namely stearic acid and triethanolamine. TEA is used as an emulsifier with stearic acid with a TEA range of 2-4% [14].

Another study describes that the cream based on stearic acid and triethanolamine is more stable during

storage. Apart from having a soft texture, and has good homogeneity[15,16]. Therefore, in this study, 50% methanol extract from sea snails (*OnchidiunTyphae*) was formulated as cream with variations in the emulsifier. The formulations with various emulsifier bases of triethanolamine and stearic acid were made into eight formulas. Range of stearic acid at 16-18% and a concentration of triethanolamine in the range of 2-4%. They optimized this cream extract of the Onchidiid slug formula using Design Expert 11.0.0 Trial software with the Simplex Lattice Design method using the parameters of pH, adhesion, and spreadability. The cream formed was tested for physical properties and stability, and the best antioxidant cream formula was tested for antioxidant activity using the DPPH method.

Table 1.Formula composition of OnchidiidslugExtract Cream using simplex lattice design

Code	Actual Values							
Level	X1				X2			
1	18				4			
0	16				2			
Comp	Running Code							
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
X1	1	0.25	1	0.75	0	0.5	0.5	0
X2	0	0.75	0	0.5	1	0.5	0.5	1
	Level concentration							
X1	18	16.5	18	17.5	16	17	17	16
X2	2	3.5	2	2.5	4	3	3	4

Annotation: X1: Stearic Acid; X2 Triethanolamine (TEA) Cetyl alcohol concentration 2%; liquid paraffin 2%; DMDM Hydantoin 0.02%; Glycerin 10%; oleum rose 0.5%; aquadest add 100

MATERIAL AND METHODS

Instrument:

Design Expert trial 11.0 software with the Simplex Lattice Design (SLD) instrument specifications, incubator (Memmert IN55, Schwabach-Germany),multichannel SOCOREX Acura micropipette(Switzerland), spectrophotometry (UV Genesys 10 experiment, 335903) (Thermo scientific Spectronic, United States of America), Buchi 23022A010 Rotary Evaporator (Poland),IWAKI Microplate Multi Well Plate 96 wells Flat Bottom, and Pyrex glasswere.

Materials:

Onchidiid Slug collect from Sambas waters, West Kalimantan. glycerin (Brataco no. batch 010320), DMDM Hydantoin (batch 6440-58-0), DPPH (TCIBatch no D4313), oleum rosae, liquid paraffin (Brataco batch 15B10-H11-00002), cetyl alcohol (Brataco batch C1618-20190411), triethanolamine (Brataco batch 2378-MC2). Other materials were ethanol and methanol from Merck, sterile distilled water.

Preparation of onchidiid slug methanolicextract

The fresh onchidiid slug collected from Sambas district, West Borneo waters with a dimension length of 5-7 cm. The sample was determinedat the Ecology Laboratory, Faculty of Science and Mathematics, Department of Biology, Universitas Tanjungpura. The fresh onchidiid slug was washed thoroughly to remove the impurity.The following process is the purification of the mucus. The mucus is removed by boiling for 30 minutes with constant stirring. Onchidiid slug are also clean of impurity. The meat of onchidiid slug was oven dried for 1x24 hours at 60^{o}C. The sample was pollinated by grinding the dried sample. The onchidiid slug powder is macerated with methanol solvent. The solvent was changed after 1x24 hours to obtain five filtrate solutions on days 1-3 with increasingly non-colors. The filtrate and precipitate were then filtered using a Buchner funnel and deposited on a rotary evaporator (Buchi23022A010) at a temperature of 60-65˚C to form a thick, paste-textured extract, with the color of the extract being brown. to obtain a thick extract that can be tested.

Methanolicextract cream preparation

The cream preparations were obtained based on the Design Expert 11.0.0 Trial formulation, where eight types of formulations with differentiating variables, namely TEA and stearic acid. Variable TEA and stearic acid were obtained based on the number of runs according to the program's Simplex Lattice Design (SLD) instrument. The cream formula (table1) also contains the same amount of excipient in each formula.

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Cream Formula Optimization

The optimization parameters of the cream formulation used were organoleptics, homogeneity, viscosity, spreadability, adhesion and pH. For the three parameters: Spreadability, Adhesion and pH, two SLD variables were used. The variables used are stearic acid and TEA. The optimal cream formulation was verified based on the physical stability of the preparation at day 1 and day 28[14].An organoleptic test was performed, aimed at seeing the physical appearance of the preparation by paying attention to the color, odor and shape of the formulated cream preparation[17].

The homogeneity test aims to detect changes in the cream in terms of its homogeneity. For this purpose, the cream is applied to a transparent glass and smeared in three places, including the top, bottom and center. Homogeneity is determined by the presence of coarse granules in the cream preparation[18].A viscosity test using a viscometer (Ametek Series DVEERVTJ0) was performed to determine the flow properties of a product so that it can indicate changes in the physical stability of the product. The dispersion test aims to determine the uniform distribution of the cream when applied to the skin. A good dispersibility of the cream is in the range of 5-7 cm^{2}. The cream was weighed on an analytical balance to 0.5 g and then placed on a scaled round glass. Another round jar with a transparent material and a ballast was placed on top of the cream, then waited for 1 min and then recorded the diameter of the distributed cream.

The adhesive test aims to determine the ability of the cream to adhere to the skin surface. This is expressed in seconds by weighing the cream preparation. 0.5 g is placed on a glass

object and applied, then another glass object is placed on the cream, then a 500 g load is applied for 5 minutes. An 80 g load is released so that it pulls on the bottom glass object. The parameter recorded is the time it takes for the two glass objects to detach. Adhesion is calculated in seconds (second)[17]. The pH test aims to check the acidity of the preparation. The preparations meet the standard criteria for the skin in a range of 4.5-6.5. The pH test is performed using a pH meter, where the tip of the pH conductor is dipped into the cream sample and then waiting for a while until the results of the value appear on the screen of the pH meter.

Antioxidant activity assay

The sample to be tested goes through several steps to determine its antioxidant activity. These steps include: Preparation of a DPPH standard solution by carefully weighing (Ohaus Pioneer PX224/e) the material to obtain a concentration of 39.4 ppm, which is dissolved in a pro-analytical methanol. Then the standard is prepared in series of 0.020, 0.040and0.060 mM concentration with 3 replicates. The prepared standard is then measured as the maximum wavelength of the solution (39). Thus, a maximum wavelength of 515.7 nm was obtained using a UV-VIS spectrophotometer (Shimadzu UV-2450 series). The second step is to determine the IC_{50} value in the samples of methanol extract and cream preparations containing the samples and compare with the standard solutions of vitamin C.

RESULTS AND DISCUSSION

Determination sample

Determination is the primary and initial process in conducting research and determining the characteristics of a particular species. Based on the determination results, it is known that the species of the sample classification belongs to the species *Onchidiumtyphae*. The identification results were obtained at the Faculty of Mathematics and Natural Sciences, UnivertasTanjungpura.

Preparation result

The net weight of the prepared extract paste was 14.5 g, the weight of the simplicia used was 160.22 g, and the percentage yield of the methanol extract of onchidiid slug was 9.05% (figure 1). Organoleptically, the cream has a distinctive smell of sea slug, a slightly salty taste and is brown in color; this corresponds to the results of the onchidiid slug extract of *Discodorissp*, which according to the research of the scientific journal Hafiluddin and Nurjannah obtained a thick extract with a characteristic brown color[8]. The results of the physical assay of the cream preparation are shown in table 1.

F1 F2 F3 F4

F5 F6 F7 F8

Figure 1. The cream form of the methanol extract at various formulas.

Table 2. Results of Physicochemical for Onchidiid slug Extract in cream dosage form

Formula	Physicochemical Parameter											
	Spreadability (cm^2)				Adhesive Strenght (Sec)				pH			
	Day-1		Day-28		Day-1		Day-28		Day-1		Day-28	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
F1	3.51	0.26	3.78	0.12	41.73	1.29	40.83	1.89	6.23	0.15	6.26	0.15
F2	5.72	0.01	5.49	0.15	29.52	2.02	29.99	2.56	5.20	0.10	5.30	0.10
F3	3.55	0.23	3.30	0.22	41.75	1.28	41.38	3.95	6.24	0.05	6.20	0.10
F4	3.40	0.11	3.80	0.26	40.41	2.52	40.42	1.90	5.53	0.15	5.70	0.10
F5	7.20	0.15	6.63	0.05	18.52	1.22	19.51	1.43	6.03	0.37	6.40	0.20
F6	4.56	0.20	5.01	0.35	33.22	2.75	35.93	1.99	6.36	0.75	6.26	0.58
F7	4.45	0.16	4.60	0.07	32.35	0.83	33.85	0.68	5.90	0.60	6.03	0.20
F8	7.41	0.21	6.91	0.21	19.57	0.86	18.91	0.55	6.22	0.11	6.36	0.20

Cream Formula Optimization

The organoleptic results (Figure 2) showed that the preparation of onchichiid slug extract cream had a soft, yellowish-white texture and a distinct onchichiid slug aroma on day 1, and soft, yellowish-white and with a characteristic aroma of sea slug on day 28.As a result of the homogeneity test, the preparations showed good homogeneity of the cream on the first day and on the 28th day, which can be attributed to the use of a formulation with a homogeneous base characterized by the absence of coarse grains on the slide (Table 2). The test results also show that there is no separation between the oil phase and the water phase. This result is due to the presence of stearic acid and TEA, which act as an emulsifier, where the emulsifier is an emulsifier that can reduce the interfacial tension between oil and water and surrounds the droplets of the dispersed substance to prevent coalescence from occurring and the dispersed phase from separating[19]. The tendency of the preparation to be more stable may also be caused by the composition of the dominant stearic acid, due to the higher conversion of stearic acid (decreased amine ester acid value) along with the increase of Al content (decreased Si/Al ratio) to 25 Si/Al ratio. The conversion no longer increases[20].

A Brookfield brand Ametek viscometer was used in this study, employing size 6 and 7 spindles. Viscosity tests were conducted using the formula recommended by Design Expert 11.0 Trial in the method SLD, namely the formula with a ratio of 16.16% stearic acid and 3.84%. The results for

viscosity with spindle 6 at speeds 2, 4, 6, 10, 20 rpm are 9673±3.1977 cP and with spindle 7 134551±166.36 cP. The unit cP (centipoise) is a unit describing the dynamic viscosity value in the centimeter unit system. Rheological analysis was performed to study the flow of liquids and the deformation of solids. Rheology aims to characterize the product, which affects the physical stability and bioavailability of the preparation[21–23]. The results of viscosity curve and shear stress versus shear rate show pseudoplastic flow, where pseudoplastic flow shows that the greater the given shear stress (force), the lower the viscosity (Pratasik et al., 2019). The nature of viscosity is related to the additive used in the cream, namely stearic acid as a base. The physicochemical properties of stearic acid, a saturated fat in the form of solid wax, tend to be stiff at room temperature, so they affect the density of the cream preparation[24].

The spreadability test was performed to test the ability of the cream preparation to spread on the skin, which is related to the spreading power of the active ingredient, namely the onchidiid slug methanol extract contained in the cream preparation. Sharon, et al., 2013). In Table 2, it can be seen that F8 has the highest dispersion value (7.41 cm2). This highest dispersion was obtained with a concentration of 16% stearic acid and 4% TEA. These results illustrate that increasing the concentration of TEA can decrease the consistency of the preparation, making it more flowable. This spreadability may be caused by dimerization between stearic acid with another stearic acid and an increase in pH by adding TEA to form a more stable mass [25,26]. Increasing the concentration of TEA also decreases the viscosity of the preparation. Below is the plot of TEA and stearic acid against dispersion in Figure 2.

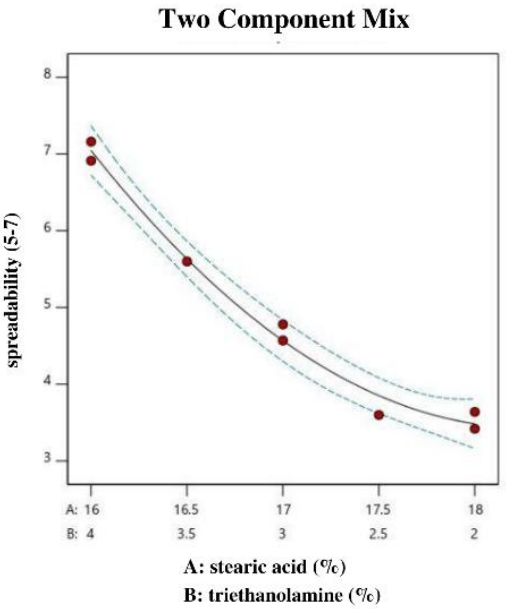

Figure 2. The curve of TEA and stearic acid on the spreadability of the preparation of onchidiid slug cream extract

Variations can affect the spreadability of triethanolamine and stearic acid in cream preparations. Based on the curve above, the plot in Figure 2 shows that the addition of TEA and stearic acid slightly increases the spreadability of the cream. The polynomial equation for the dispersion reaction is shown in Equation 1, namely:

Y = 3.48 A + 7.05 B - 2.79AB…….. (1)

Y= response obtained, (A) = triethanolamine, (B) = stearic acid, AB = combination of stearic acid and TEA proportions, AB(A-B) = combination and difference of stearic acid and TEA, AB(A-B)2 = combination and the difference between the squares of stearic acid and TEA.

Based on the equation of the combination of TEA and stearic acid according to the SLD approach, on the adhesion reaction, stearic acid has an effect compared to TEA; it seems that based on the results of the analysis, there is a significant difference (P≤0.05), indicating that variations in the concentration of TEA and stearic acid have a significant effect. Real. Stearic acid can increase the consistency of the cream and make the cream appear stiffer, resulting in increased adhesion to cream preparations[27]. Formulas F5 and F8 with a ratio of stearic acid and triethanolamine (16:4) have the highest dispersion value. This highest dispersion is due to the fact that the more stearic acid is used, the higher the viscosity; in contrast, the less stearic acid is used, the lower the viscosity. The less stearic acid used, the lower the viscosity and the higher the dispersibility. The dispersion value for preparations with natural ingredients is in the range of 9-13 cm^2.

Adhesiveness test result shown thespreadability test was performed to determine the stickiness of the cream preparation on the skin. In terms of the spreading power of the active ingredient, namely the onchidiid slug methanol extract contained in the cream preparation, it showed the expected adhesion results, which followed the criteria for good adhesion, namely 2-300 seconds. The data in Table 2 show that at F5, when the concentration of TEA is the highest and that of stearic acid is the lowest, the value of the lowest adhesion strength is present. In contrast, the value of the highest adhesive force is shown at F1 and F3, where the concentration of stearic acid is the highest and TEA is the lowest; low viscosity values cause this factor. In addition, the value of adhesion is a crucial factor in ensuring product quality, as it affects the ability of preservatives to maintain the quality of their preparations in situ. The acceptable range of adhesion force is 30.0-50.0 g/sec. Below is the curve of TEA and stearic acid for adhesion in Figure 3.

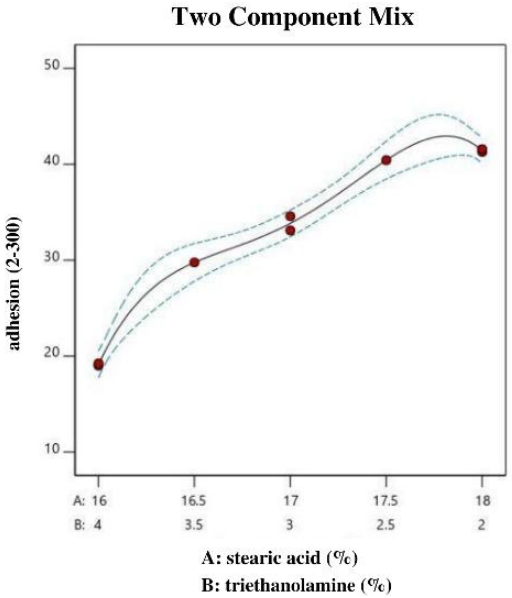

Figure 3. The curve of TEA and stearic acid on the adhesion cream of extract Onchidiid slug preparations

The adhesion curve diagram in Figure 2 shows that the higher the triethanolamine content and the lower the stearic acid content, the lower the tack; on the other hand, the more stearic acid is added, the higher the tack. The presence of low concentrations of TEA and high stearic acid results in low viscosity, so the tack obtained is low[28]. The polynomial equation for the dispersion behaviour is shown in Equation 2, namely:

Y = 3 Y = 41.42 A + 19.13 B + 14.25 AB - 2.61 AB(A-B) + 45.76 AB(A-B)2…….. (2)

Y= response obtained, (A) = triethanolamine, (B) = stearic acid, AB = combination of stearic acid and TEA proportions, AB(A-B) = combination and difference of stearic acid and TEA, AB(A-B)2 = combination and the difference between the squares of stearic acid and TEA.

Based on the equation 2 combination of TEA and stearic acid according to the SLD approach, the reaction of TEA component gave the most remarkable effect, making a significant difference ($p \leq 0.05$) on the dispersion as evidenced by the research of Gyawali et al. where the addition of TEA can reduce the consistency of the cream, making the cream thicker than the low concentration of it. Dilute and causes an increase in dispersion. The adhesion test (Table 2) for formula 1 (F1) and formula 2 (F2) gave the highest value compared to the other formulas. This adhesion is due to the fact that the less TEA and the more stearic acid is added, the higher the stickiness of the cream[28].

The onchidiid slug tested in this study was collected from the waters of Sambas, West Kalimantan, with the appropriate length. The sample is to choose the right size (5-7 cm)and fresh to get the maximum active composition.The moisture content of onchidiid slug powder was measured through a moisture balance. The result of measuring the water content was 5.37%.This result meets the requirements where the good water content is≤10%. The result allows the onchidiid slug powder to be resistant during storage to fungi and other microorganisms.

The pH test was performed to determine the acidity of the cream preparation. Regarding the pH performance of the active ingredient, namely the methanol extract of onchidiid slug contained in the cream preparation, it showed the expected pH results, which were below the criteria according to the skin conditions, which ranged from 4.5-5.6.

From the data (Table 2), the lowest pH was obtained at stearic acid concentration of 16.5% and TEA at 3.5%; this indicates that high stearic acid concentration can maintain the stability of pH for hydrolysis by adding TEA to the solution. The pH also indicates the acidity of the preparation; for cream preparations, the acceptable acidity is 5.6 -7.2. This condition serves not only to preserve the stability of the preparation from the hydrolysis mechanism due to the presence of TEA, but also to ensure that a cream is adapted to the pH of the skin. Below you can see the curve of TEA and stearic acid against pH in Figure 4.

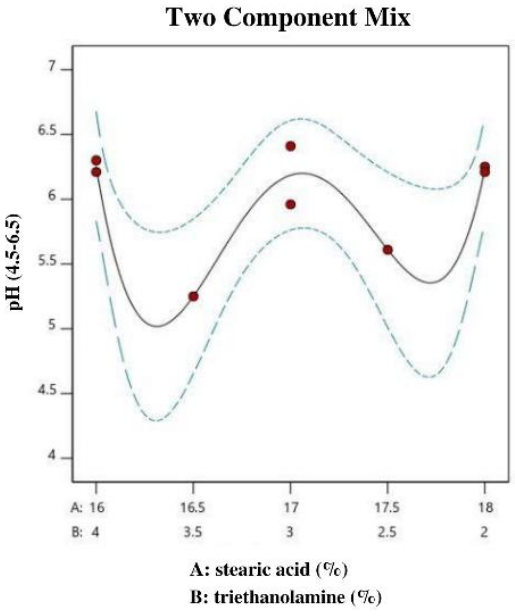

Figure 4. The curve of TEA and stearic acid on the pH of the preparation of Onchidiid slugcream extract

The shape of the above curve is not linear, which shows triethanolamine and stearic acid; both components have no significant effect on the pH of the cream preparation. This is also evidenced by the results of the ANOVA analysis, which show that the quartic equation model gives a nominal value with a "Prob-F" value greater than 0.05, and this shows that the stearic acid and pH variables have no significant effect on the pH response. The polynomial equation for the dispersion response is shown in equation 3, namely:

Y = 6.23 A + 6.25 B - 0.23 AB + 1.99 AB(A-B) - 16.41 AB(A-B)2…….. (3)

Y= response obtained, (A) = triethanolamine, (B) = stearic acid, AB = combination of stearic acid and TEA proportions, AB(A-B) = combination and difference of stearic acid and TEA, AB(A-B)2 = combination and the difference between the squares of stearic acid and TEA.

Based on equation 3, the combination of TEA and stearic acid according to the SLD approach, In the pH response analysis, TEA gives a slightly more significant effect; this is due to previous research regarding the optimization of cream formula of katuk leaf extract (*Sauropusandrogynus*) variations in the concentration of stearic acid and triethanolamine, where the content TEA compounds, the base groups make the pH of cream preparations higher. The pH test (Table 2) of formula 5 (F5) and formula 8 (F8) gave the highest value compared to the other formulas. This pH value is due to the fact that the higher the concentration of stearic acid, the higher the pH value. The results show that F5 and F8 have a pH of 6. This is influenced by the fact that the more stearic acid is contained in stearic acid due to the large number of acid groups, the lower the pH, while the more triethanolamine is contained, the higher the pH, which is due to the base groups contained in triethanolamine.

Determination of Optimal Formula Based on Simplex Lattice Design

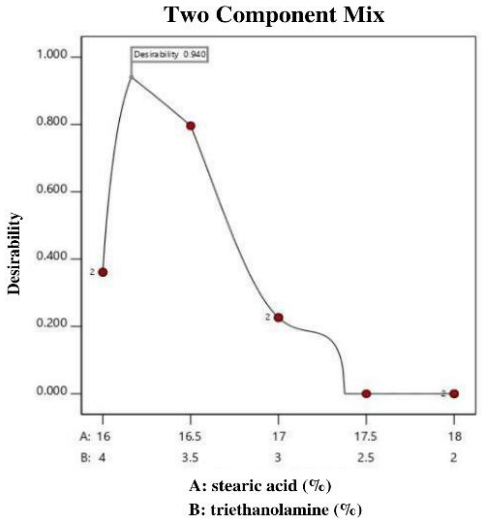

Figure 5. Optimal Cream Desirability Curve Combination of Stearic Acid and TEA

Figure 5 on the page shows the peak trend of desirability scores for the combination of components A and B. The desirability values become the benchmark for determining which components A and B represent the optimum and have optimal physicochemical properties. The predicted optimum formula consisted of 16.16% stearic acid and 3.84 triethanolamine, with a desirability value of 0.940. The predicted formula results have a stickiness of 24.576 seconds, spreadability of 6.546 cm2, and pH of 5.25. The

desirability value is the optimization target value with a range of 0-1. The regression data of the three variables have a p-value < 0.05, indicating a significant value.The next test is a normality test with The Shapiro-Wilk test with p-value > 0.05 (p-value requirement > alpha 0.05) met the normality assumption. The autocorrelation test with Durbin Watson (dwtest) with p-value > 0.05 (p-value requirement > 0.05) indicates that the p-value is above alpha and thus there is no autocorrelation. Then the homogeneity test with the Breusch-Pagan test (bptest) shows the p-value > 0.05 (more than alpha), then we can say that the variant is homogeneous.

Table 3. Result of antioxidant activity extract and cream onchidiid slug

Dosage Form	Equation	IC_{50} (ppm)
Methanol extract of onchidiid slug	y = 2.4356x + 51.675, R^2 = 0.9542	92.045
Cream of onchidiid slug	y = 11,005x + 69,72, R^2 = 0,9885	54.584
Vitamin C	y = 2,6013x + 85,278, R^2 = 0,9624	55.930

DPPH (2,2 Diphenyl 1 picrylhydrazyl) is a free radical compound that can react with antioxidants and form 1,1 diphenyl-2-picryl hydrazine compounds; the presence of DPPH compounds which are paired electrons in a DPPH compound can provide absorption wavelength in quantity=517 nm. The presence of a reaction between DPPH and the solvent is indicated by the purple color and will react to a yellow color if it reacts with compounds containing antioxidants. Some advantages of the DPPH method include storage stability which can be stored for a long time, and the DPPH method can be easily observed for changes. Antioxidant compounds will push one electron on unstable free radicals so that these free radicals will be neutralized and no longer interfere with metabolic pathways in the body. The antioxidant activity can be known based on the IC_{50} value; the smaller the IC_{50} value, the greater the antioxidant. The initial stage of nature Preparation of a 39.4 ppm DPPH solution (BM DPPH=394.32) was carried out by dissolving 3.94 mg of DPPH, diluted with 100 mL of methanol added to the limit mark so that a solution with a concentration of 39.4 ppm was obtained. The absorption of the solution was measured spectrophotometrically at a maximum wavelength of 39.4 ppm DPPH solution.

DPPH compound DPPH (2,2 Diphenyl 1 picrylhydrazyl) is a free radical compound that can react with antioxidants and form 1,1 diphenyl-2-picryl hydrazine compounds; the presence of DPPH compounds which are paired electrons in a DPPH compound can provide absorption wavelength in quantity = 517 nm. The presence of a reaction between DPPH and the solvent is indicated by the purple color and will react to a yellow color if it reacts with compounds containing antioxidants. Some advantages of the DPPH method include storage stability which can be stored for a long time, and the DPPH method can be easily observed for changes. Antioxidant compounds will push one electron on unstable free radicals so that these free radicals will be neutralized and no longer interfere with metabolic pathways in the body. The antioxidant activity can be known based on the IC_{50} value; the smaller the IC_{50} value, the greater the antioxidant activity. The initial stage of nature Preparation of a 39.4 ppm DPPH solution was carried out by dissolving 3.94 mg of DPPH, diluted with 100 mL of methanol added to the limit mark so that a solution with a concentration of 39.4 ppm was obtained. The absorption of the solution was measured spectrophotometrically at a maximum wavelength of 39.4 ppm DPPH solution.

Determination of the maximum wavelength on the measurement of the DPPH wavelength with a concentration of 39.4 ppm (1 mol) shows a wavelength of 515.70 with an absorbance of 1.018. Testing of antioxidant activity using the DPPH method on cream preparations was carried out with the initial step of preparing cream samples by weighing 1 g of F1-F8 preparations, added with 10 mL of methanol solvent, then filtered and accommodated the filtrate of the sample solution that had been filtrated as much as 4 mL added to the solution. DPPH with a ratio of 1:1, then the solution mixture was incubated for 30 minutes in a dark glass bottle. The absorbance was measured using UV/VIS spectrophotometry with a maximum wavelength of DPPH.The results of the absorbance measurement of the vitamin C solution can be seen in Figure 6.

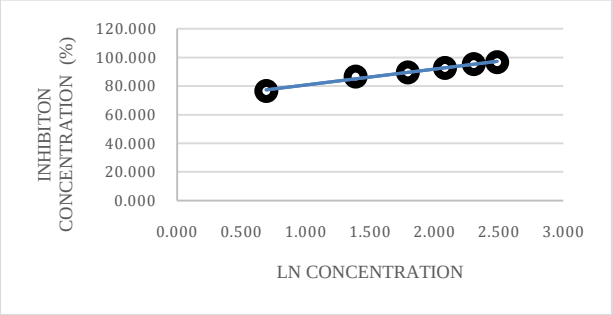

Figure 6. Graph of Vitamin CControl Absorbance measurement

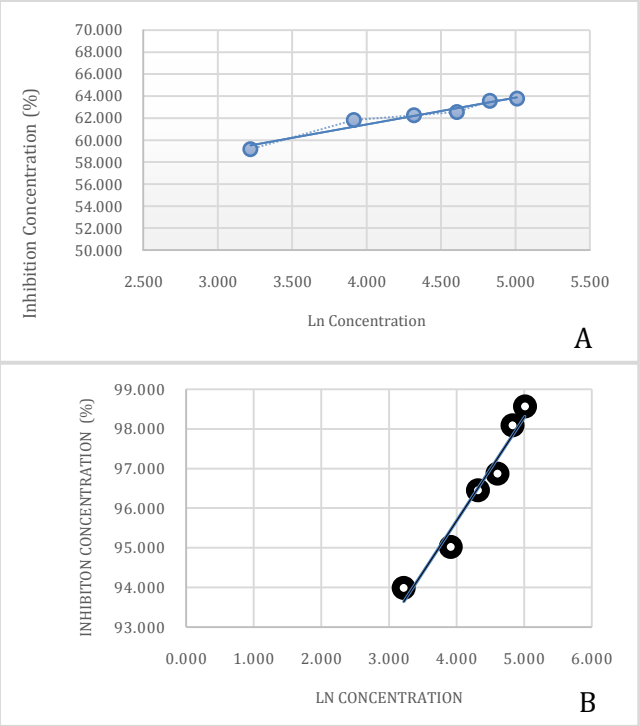

Figure 7. Absorbance graph linear regression of Extract (A) and cream Onchidiid slug(B)

Based on the antioxidant activity test (figure 7) results carried out on the preparation of slug onchidiid methanol extract, the optimum formula cream for the slug onchidiid methanol extract and vitamin C as a comparison,

respectively, were 92,045 ppm, 54,584 ppm, and 55,930 ppm where the three antioxidants were categorized as potent antioxidants, which has a value of 50-100. This antioxidant value proves the presence of solid antioxidant compounds in the extract samples and Onchidiid slugextract cream. Antioxidant cream preparations have the most significant DPPH free radical scavenging activity compared to the IC_{50} values of extracts and vitamin C (comparison). This antioxidant value happens in addition to the effect of the antioxidant content in the extract, the presence of components of the base contained in the cream, which has a hydroxy group, in which the atom (H_3O^+) containing one proton in the hydroxyl group will react with the unstable electron atom (free radical) of the extract [29]. DPPH. This hydronium ion causes the DPPH free radical reaction, which will be stable and become a DPP hydrazine compound.

CONCLUSION

The formula of cream methanol extract of sea snails with a ratio of 16.16% stearic acid and 3.84% triethanolamine is optimal for it by producing an antioxidant cream formulation that has been tested physically and for its stability. The formula with a ratio of 16.16% stearic acid and 3.84% triethanolamine is the optimal formula for Simplex Lattice Design (SLD) with optimum physicochemical properties with adhesion value of 24.576 seconds, dispersion of 6.546 cm2, and pH 5.25. The antioxidant activity test showed that the IC_{50} of the extract in the cream was higher than the single extract; this can illustrate that the preparation of Onchidiid slugcream has the potential as an antioxidant.

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Ethical Approval:
This research has passed the ethical clearance with No.700/UN22.9/PG/2022.

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Conflict Of Interest:
The author declares that there is no conflict of interest and all have an equal share in the content of this article.

Informed Consent: The research focused on antioxidant preparation base on local wisdom Borneo Island, Onchidiid Slug (*Onchidiiumtyphae*).

Authorship

B. Wijianto: main idea of study, writing manuscript, extraction sample, final approval, data analysis
A. Fahrurroji :data analysis, writing manuscript, final approval
I.G. Kemuning :writing manuscript, extraction sample, final approval, data analysis

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