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2 *Chlorella sp* : Extraction of fatty acid by using avocado oil as solvent and its application as an anti-aging cream

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2 **Abstract.** The study aimed to analyze the fatty acid content of *Chlorella sp* crude extract by using avocado oil solvent and determining the effectiveness of fatty acids *Chlorella sp* as the anti-aging cream. The extraction of fatty acids from *Chlorella sp* using avocado oil as a solvent with three ratios were 1:10, 1:20 and 1:25 w/V. The highest lipid content was obtained at 1:20 w/V (gram microalgae: mL avocado oil) yielding 52.73%. Crude extracted were analysis by GC-MS and FTIR, and skin condition was determined by skin analyzer. The effectiveness test of *Chlorella sp* cream was applicated on the face of the panelists aged 20-60 years. From 10 panelists, the applied of *Chlorella sp* cream was 90% increased on the facial skin yielded moisture and oil content, 70% repair the skin structure. The composition of fatty acids *Chlorella sp* extract was palmitic acid, linoleic, oleic and stearate. Fatty acids crude extract of *Chlorella sp* can improve the effectiveness of anti-aging cream. The cream from *Chlorella sp* was more effective than the cream without containing microalgae. This is very promising because it is alternative to organic solvents i.e. green chemistry.

1. Introduction

Human skin will dry up when aged 25 years and over. This is due to the dense activity, UV exposure, pollution, and cosmetic usage, resulting in the decrease of oil gland and sweat production on the skin, with the result that the water within the skin becomes more volatile [1] Moreover, the factor of increasing age will reduce the thickness of the dermis layer. This condition occurs which does elastin and collagen fibres is loss in the skin [2]

Many research studies have found that ingredient in anti-aging cream is a moisturizing. Moisturizers are a product designed to restore and maintain optimal hydration at the stratum corneum. Moisturizers contain lipids such as triglycerides and mineral oils [3].

Currently, the main producers of fatty acids are marine microorganisms that are microalgae such as phytoplankton. The phytoplankton synthesizes omega-3 and omega-6 fatty acids which include EFA (Essential Fatty Acid) and DHA (Docosahexaenoic acid). EFA and DHA serve as an energy source, maintain the body heat loss, prevent skin from drying and peeling and protect tissues and other organs [4].

Phytoplankton contained fatty acid were needed by human skin such as Palmitic Acid, Stearate acid, Oleic acid, and Linoleic acid. Phytoplankton also possesses several advantages that are abundant



as natural resources, not dependent on climate and weather, fast-growing time that can be harvested not in the long-term period. In addition, phytoplankton can be produced continuously, causing no adverse impact on the environment, its production might be controlled according to the need and desire, and safety for health [5].

One of the phytoplankton that has an active component is *Chlorella sp* which possesses a fatty acid content approximately 31% - 68% [6]. *Chlorella sp* is the type of phytoplankton that has commercial potential that had been used to produce fats, fatty acid, proteins, and pigments [7].

The common solvent employed in the production of anti-aging cream is a toxic organic solvent so that the need for a safe alternative solvent used for the skin. Human skin contains fatty acid palmitoleic, palmitate, stearate, oleate, linolenic and linoleic which is also contained in avocado oil [8][9]. So that avocado oil can be used as a natural solvent that can be used to extract fatty acids from phytoplankton by sonication method [10] with a heating temperature of 50-60°C [11].

Based on the description of the background, it will be conducted the research on "Extraction Fatty Acid of *Chlorella sp* with Avocado Oil and Its Application as an Anti-Aging Cream".

3. Material and Methods

The materials used in this research work include *chlorella sp* powder (Production of Laboratorium Pakan Hidup Laboratory, Balai Besar Perikanan Budidaya Air Payau Jepara), avocado oil (Production Healthy Organic Store) methanol pa, stearic acid ($\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$), glycerol, triethanolamine ($\text{HOC}_2\text{H}_4)_3\text{N}$), nipagin, Nipazol, propylene glycol, DPPH, vitamin C pa (Production of PT Brataco obtained from CV Sumber Rejeki), aquadest, aquabidest (Production Science Building FMIPA UNHAS), filter paper, label paper, aluminum foil and tissue roll.

The apparatus used in this research work included glass tools which are generally used in the laboratory, centrifuge, digital scales, mortar and pestle, plastic pot, Erlenmeyer, pipette, Brookfield viscometer, pH meter, pycnometer, ultrasonic Cleaner Krisbow 42 kHz, spectrophotometer, UV-Vis US-110PC, *Skin analyzer Luvance*, FTIR (*Fourier Transform Infra Red*) Prestige-21 Shimadzu, and GC-MS QP2010S SHIMADZU.

2.1. Extraction Lipid of *Chlorella sp*

The powder of *Chlorella sp* was added to Erlenmeyer 50 mL, then added avocado oil with various ratios (g microalgae: mL of avocado oil) 1:10 w/V (weight/Volume), 1:20 w/V, 1:25 w/V. The mixture sonicated for 6 hours with temperature range 50-55°C. The mixture was added aquadest and shaken. The oil and aquadest were separated by centrifugation. The obtained lipids analyzed using FTIR (*Fourier Transform Infra Red*) Prestige-21 Shimadzu, and GC-MS QP2010S SHIMADZU to know the fatty acid content.

2.2. Production of *Chlorella sp* Cream

Preparation of creams was separated into two phase are oil phase (avocado oil, and microalgae extract, stearic acid, triethanolamine) and aqueous phase (aquadest, nipagin, nipazol).

2.2.1 *Chlorella sp* crude extract is dissolved with stearic acid at 60-70 °C (oil phase) and added Triethanolamine.

2.2.2 Nipagin and nipazol were weighed and dissolved in propylene glycol (water phase).

2.2.3 Oil phase was moved to a hot mortar and added gradually aquadest while stirring until creamy.

Chlorella sp cream then tested according to SNI 16-4399-1996 is the organoleptic test, pH, density, viscosity and microbial contamination. *Chlorella sp* cream effectiveness test was carried out randomly to 10 panelist aged 20-60 years, and one panelist as a control. Skin Analyzer was used to analyze the texture facial skin of panelist before and after the use of *Chlorella sp* cream and control cream.

2.3. Antioxidant Activity Test of *Chlorella sp* Cream and Avocado oil Cream (Control Cream)

25 mg *Chlorella sp* was dissolved in methanol pro analysis. Standard series in various concentrations 20, 40, 80, 160 and 320 ppm. As much as 0.01; 0.02; 0.03; 0.04 and 0.05 mL put in volumetric flask and added 1 mL of 0.4 mM DPPH solution in methanol pro analysis. The solution was shaken and stored at room temperature for 30 min, after that, the absorption was measured at a maximum wavelength. Vitamin C was utilized for a positive control and for a reference. Antioxidant activity is expressed by percent of DPPH immersion.

3. Result and Discussion

3.1. Extraction Lipid of *Chlorella sp*

The most lipid result obtained from microalgae crude extract of *Chlorella sp* with avocado oil was at ratio 1: 20 with the percentage to 52,73% .

Table 1. Result of Isolation Lipid *Chlorella sp*.

<i>Chlorella sp</i> (gram)	Sample		Extract	
	Avocado oil (mL)	Dry Residue Sample (gram)	Extracted Sample (gram)	Lipid Sample (%b/b)
1,15	10	0,89	0,26	22,61
1,10	20	0,52	0,58	52,73
1,12	25	0,76	0,36	32,14

3.2. Characteristics of Avocado Oil and Lipid of *Chlorella sp* with Gas Chromatography - Mass Spectrometry (GC-MS)

The content of avocado oil and lipid of *Chlorella sp* crude extract based on the dominant peak was shown in Table 2. The dominant number of fatty acids identified of avocado oil were 7, 8, 9, and lipid of *Chlorella sp* crude extract were 1, 3, 5. The result of the dominant peak was compared to database WILLEY229.LIB.

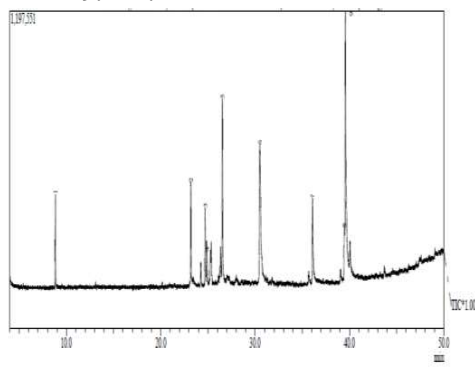


Figure 1. Avocado Oil Chromatogram.

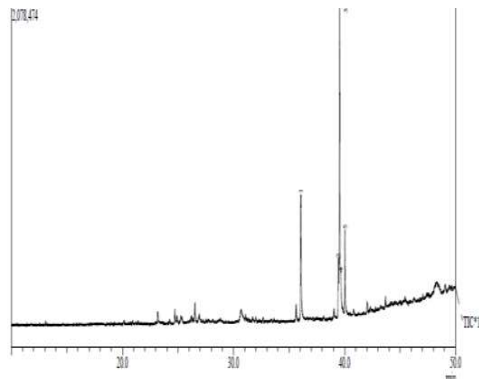


Figure 2. Lipid extract of *Chlorella sp* with avocado oil.

Table 2. Composition of Avocado Oil and lipid of *Chlorella sp* crude extract with avocado oil

Fatty Acid	Avocado Oil (%)	Lipid of <i>Chlorella sp</i> crude extract (%)	Fatty Acid Decrease (%)	Fatty Acid Increase (%)
Palmitic acid	6,64	23,07	-	16,43
Linoleic Acid	3,57	8,04	-	4,47
Oleic Acid	32,73	56,73	-	24
Stearate Acid	-	12,16	-	12,16

The GC-MS analysis of avocado oil showed the presence of 9 peaks, with the highest abundance existed at the 9th peak with a retention time of 39.567 minutes. The lipid of *Chlorella sp* crude extract exhibited 5 peaks with the highest abundance also at the 3rd peak with retention time 39,553. Based on WILLEY229.LIB databases, peak 9 and 6 referred to octadecanoic acid or known as oleic acid. This compound had MW at m/z 296. Oleic acid content on avocado oil was 32,573% and lipid of *Chlorella sp* crude extract was an enhancement to 56,73%.

Oleic acid is the most dominant compound in the composition of the avocado oil. Oleic acid also has a function as an antioxidant [12].

Peak number 7 with a retention time of 36.093 minutes for avocado oil and peak number 1 for Lipid of *Chlorella sp* crude extract with a retention time of 36.060 referred to a hexadecanoic acid compound known as palmitic acid. This compound had MW at m/z 270. This compound in avocado oil before sonication levels was 6.64% and after sonication reached 23.07%.

The third dominant peak with a retention time of 39.458 minutes for avocado oil was the number 8 peak referring to the 9: 12 octadecanoic acid compound or known as linoleic acid. This compound possessed molecular weight at m/z 294 with a compound content reached 3.57%. And peak number 5 for Lipid of *Chlorella sp* crude extract with a retention time of 39.967 referred to Octadecanoic acid compounds or known as stearic acid. This compound had molecular weight at m/z 298 with a compound content reached 12.16%

The increase and decrease of fatty acid concentration in each comparison are due to the time of sonication process the percentage of the oleic acid fraction was more detached in triglycerides than palmitic acid, linoleic acid, stearate acid, laurate acid and palmitoleate acid, so the percentage of fatty acid was reduced.

3.3. Characteristics of Avocado Oil and Lipid of *Chlorella sp* with Fourier Transform Infra Red (FTIR)

The analysis of fatty acid using by FTIR aimed to determine the functional groups presented in the sample. Figure 3 and figure 4 illustrated the IR spectrum of avocado oil and lipid of *Chlorella sp* crude extract.

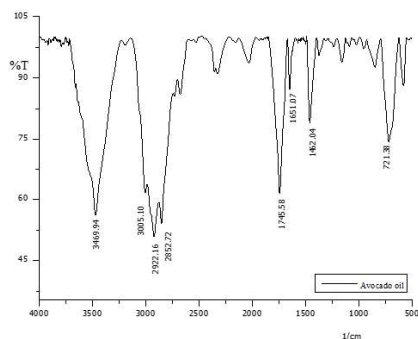


Figure 3. Spectra Avocado oil.

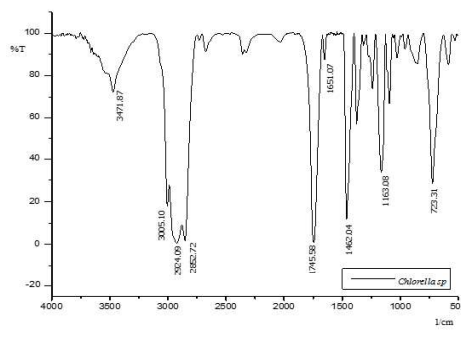


Figure 4. Lipid extract of *Chlorella sp* with avocado oil

The FT-IR spectrum of avocado oil and lipid of *Chlorella sp* crude extract exhibited an absorption range in 3469,94-3471,87 cm^{-1} indicating the presence of hydroxyl group (-OH). The wave number at 1651.07 cm^{-1} was a stretch that arose as a result of $\text{-C}=\text{C-}$. This was supported by the absorption peak at 3005.10 cm^{-1} which showed a stretching bond of $=\text{C-H}$. Furthermore, strong absorption also occurred in the wave number 2852,72-2924,09 cm^{-1} which were the stretching of the saturated carbon chain. The typical absorption of triglycerides appeared at the wave number 1745.58 cm^{-1} which indicated the absorption of the carbonyl group -C=O and supported by C-O stretching at wavenumber 1163,08 cm^{-1} , while absorption at wave number 1462,04 cm^{-1} was -C-H bending and wave number of 721,38 - 723,31 cm^{-1} was CH_2 .

Table 3. Results of FTIR Analysis Lipid Avocado Oil

Number	Wave Numbers of Avocado Oil (cm ⁻¹)	Wave Numbers of Lipid <i>Chlorella</i> sp Extract with Avocado Oil (cm ⁻¹)	Information
1	3469,94	3471,87	-OH
2	2852,72	2852,72	stretching bond C-H
	2922,16	2924,09	
3	3005,10	3005,10	-C=O
	1745,58	1745,58	
4	1651,07	1651,07	-C=C- Stretching
5	1462,04	1462,04	-C-H bending
6	721,38	723,31	CH ₂
7	-	1163,08	C-O

3.4. Antioxidant Activity Test from *Chlorella* sp Cream

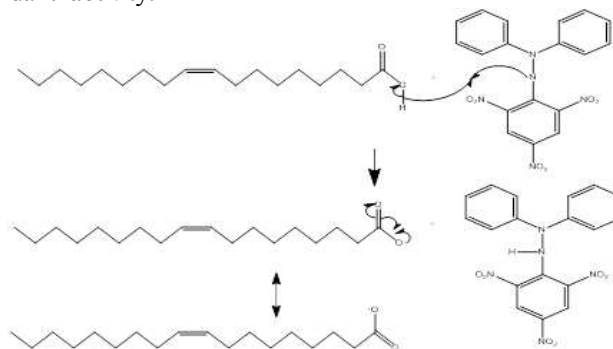
The result of antioxidant activity test of *Chlorella* sp cream (sample A), and Avocado Oil as a control cream (sample B) can be seen in table 4 below:

Table 4. Results of Antioxidant Activity Test from *Chlorella* sp cream and Avocado Oil cream

NO	Cream Sample	Value of IC ₅₀ (μg/mL)
1	A	565,330
3	B	1.103,711
4	Ascorbit acid	2,896

Based on the table 4 samples of cream preparations A and B possessed weaker antioxidant activity than ascorbic acid which had an extremely strong antioxidant activity. The smaller the value of IC₅₀ then the larger the antioxidant activity of a compound. This was because the compounds extracted from the microalgae were triglycerides that had no antioxidant activity.

The IC₅₀ value of sample A was lower than that of sample B indicating that sample A underwent an increase in antioxidant activity than sample B. Sample A was *Chlorella* sp cream and sample B was avocado oil cream as a control cream. This suggested an increase of % inhibition on sample A which was probably due to a reaction between fatty acid compounds from *Chlorella* sp extract and DPPH thus raising their antioxidant activity.

**Figure 5.** Prediction reaction of Fatty acid with DPPH.

3.5. Profile Anti Aging Cream

There are two cream, the first is cream was made from the fatty acid extract of *Chlorella sp* has pH 5,5, density is 0,967 g/cm³ and viscosity is 15,066,667 Cps. The second is cream made from avocado oil without adding the phytoplankton extract has pH 6.5, density is 0,951 g/cm³ and viscosity is 12,400 Cps. Both of creams showed the homogeneous result. Both of cream has phytoplankton smell. The use of cream does not irritate or does not cause itchy skin, does not make skin red and not rough or scaly. The results of *Chlorella sp* cream and cream control tests were still in accordance with SNI 16-4399-1996 quality standard.

3.6. Effectiveness of Anti Aging Cream Evaluation

The effectiveness test of cream was undertaken to 10 panelists with age range around 20-60 years. The results apply of *Chlorella sp* microalgae extract cream to human skin exhibited a smoother skin texture.

Control cream and cream containing *Chlorella sp* microalga were applied to the 43-year-old panelist with cream usage for one month. Figure 4 illustrated the skin texture of the use of control creams and *Chlorella sp*.



Figure 6. Control cream.



Figure 7. *Chlorella sp* cream.

The use both of cream for one month period showed result better improvement of facial skin textures. The results indicated that creams containing microalgae (figure 7) produced a better effect than no microalgae or control cream (figure 6).

The skin-analyzer tested results represented the used of creams that contained microalgae were able to enhance the water content and oil on the panelist skin. The epidermal layer especially the stratum corneum is the outer layer surface that has a balance between water and lipids to keep the skin elastic and smooth. Lipids role to maintain the natural moisture factor remains in the cell so that no excessive evaporation of water, and water contents determine the skin's moisture level [13].

Figure 8 and Table 5 illustrated a change in the facial skin of the panelists in which 90% of panelist using cream, moisture, and oil content on the face increased, and 70% of panelists experienced an improvement on skin texture.

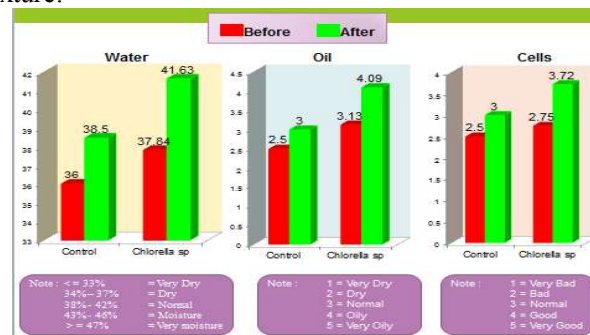


Figure 8. Graph of Facial Skin Changes Panelist.

Table 5. Percentage of Changes in Facial Skin of Panelists

Observation	Up (%)	Down (%)	Constantly (%)
Water	90	10	-
Oil	90	-	10
Cells	70	-	30

4. ¹Conclusions

Based on these results, it can be concluded that lipid extract of *Chlorella sp* content are oleic acid, linoleic acid, palmitate acid, and stearate acid. The fatty acids of *Chlorella sp* increasing of anti-aging activity.

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