Analysis of Polyphenol and Antioxidant Chlorella Vulgaris Extract: Preliminary Study

Irfan Dammar[1], Ira Tanti[2]*, Lisa R. Amir[3]

1. Doctoral Study Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
2. Department of Prosthodontics, Faculty of Dentistry, Universitas Indonesia, Indonesia.
3. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia.

Abstract

Microalgae is an alternative material that can be used for various application in dentistry. Type of microalgae that is easy to obtain is Chlorella Vulgaris which is known have a high protein contents that are useful in forming the new tissues. The present study aimed to analyze the content of polyphenols, antioxidants, and the physical stability of Chlorella Vulgaris gel. Microalgae was collected from sea water, followed by sterilization and screening process to obtain Chlorella Vulgaris seeds. Seeds culturing and extraction was carried out to obtain powder, and then tested for polyphenol content and examined antioxidant activity. Physical stability tests included the dispersion, pH, and viscosity tests of the Chlorella Vulgaris extract gel. one-way and two-way ANOVA were utilized to analyse the data.

The results of the phenol content test showed that the antioxidant activity was 0.087% in the chlorella Vulgaris extract. The results of the physical stability test showed a spreadability of 3.9 cm with a pH of 9, and a semisolid, pseudoplastic gel viscosity. Chlorella Vulgaris contains high antioxidant concentration and activity, thus appropriate physical stability to be used to accelerate tissue regeneration.

Experimental article (J Int Dent Med Res 2023; 16(1): 149-153)

Keywords: Chlorella vulgaris, phenolic, antioxidant, physical stability.

Received date: 11 January 2023 **Accept date:** 03 February 2023

Introduction

The development of biomaterials using microalgae is an option that needs to be considered in the medical world, due to its ability to provide a biological response with maximum effect on the body[1] Microalgae are natural materials consisting of unicellular eukaryotic cells which are rich in primary and secondary active components[2,3]. The molecular content of microalgae has anti-inflammatory, antibacterial, antifungal, and antioxidant effects needed in health biotechnology[4].

Chlorella Vulgaris is a microalga that is easily found in seawater and can be cultivated on a large scale[5]. Its ability to grow rapidly under autotropic, mixotrophic, and heterotropic conditions makes it capable of being cultivated easily[6,7]. A single Chlorella Vulgaris can form colonies of up to 64 cells and has high photosynthetic ability, and has antibacterial properties due to the presence of unsaturated lactones, cyanogenic glycosides, sulfur components, phenols, phenolic glycosides, saponins, and phytoalexins[3,5,6].

Chlorella Vulgaris is expected to be able to help fibroblasts to produce extracellular matrix protein structures, such as laminin and fibronectin, as well as glycosaminoglycan basic substances such as hyaluronan and glycoprotein. Production of other extracellular matrices such as adhesive proteins and basic components of glycosaminoglycan and proteoglycan filling. Biologically, fibroblasts play a role in helping wound healing, inflammation, and angiogenesis. The presence of high protein content in chlorella Vulgaris can help increase the production of extracellular matrix protein structures. The secretion of cytokines in the inflammatory process will guide the activation and migration of immune cells, which then the basic ingredients of the extracellular matrix play a role in the extravasation of immune cells into connective tissue. A study reported that the use of chlorella Vulgaris accelerated wound healing with minimal scarring, due to the presence of beta carotene

Corresponding author:
Ira Tanti
Department of Prosthodontics, Faculty of Dentistry, Universitas Indonesia, Indonesia
E-mail: iratanti@ymail.com

and various vitamins that help form collagen fibers and intracellular matrix[8,9].

The content of polyphenols in microalgae affects antioxidant activity in wound healing, this is due to the presence of phenol, ethanol, and flavonoids which can neutralize free radicals by donating electrons or hydrogen atoms[8,9]. Several studies have reported that phenolic content and antioxidant capacity have a linear relationship and that flavonoids are known for their antioxidant properties, thereby providing a free radical scavenging effect and increasing wound healing[9,10]. One that influences the magnitude of the antioxidant activity of polyphenols is the number and arrangement of the hydroxy groups, the type of treatment applied, and the culture conditions[11,12.]

The process of wound healing a process that depends on many factors, one of which is by increasing the content of protein and collagen which helps fibroblast cell migration and extracellular matrix synthesis due to antioxidant activity[8]. Several other studies have reported the strong antioxidant activity of various plants shown through inhibition of lipid peroxidation and increased catalase activity[13]. The presence of antioxidant enzymes such as superoxide dismutase and catalase contributes to counteracting free radicals that inhibit tissue regeneration[8].

One way to increase the effectiveness of using chlorella Vulgaris is to formulate chlorella Vulgaris extract in gel form. In previous studies using chlorella Vulgaris extract cream, it was stated that a 5% cream content gave good results for wound healing, so it was necessary to test it in gel dosage form[14]. Gels are semi-solid systems consisting of suspensions made up of small inorganic particles or large organic molecules, interpenetrated by a liquid[15]. Testing for phenolic compounds in chlorella Vulgaris is needed to determine the total phenol levels that can affect the antioxidant activity and to make extract preparations in gel form. Therefore, this study reports the phenolic content, antioxidant activity, and physical stability of the chlorella vulgaris extract gel.

Materials and methods

Cultivation of chlorella vulgaris

The chlorella Vulgaris microalgae were taken from the sea on Galesong beach using a planktonic to obtain as much as 75 gr. Seawater was brought to the laboratory of the Galesong Brackish Water Cultivation Center, to analyse the types of phytoplankton found. The chlorella phytoplankton was then placed in agar media with added fertilizer. Mose needles that have been sterilized by the heating method, the agar media was streaked into an Erlenmeyer tube that has been filled with 10 ml of sterile seawater. Seawater must go through the chlorine test stage. If seawater was found to be neutral, the seawater was then filtered with cotton and then sterilized with an autoclave.

Preparation of chlorella vulgaris extract

Chlorella vulgaris seeds were cultured by mixing 10 ml of chlorella seeds into 200-300 ml of sea water. Inside the Erlenmeyer tube and given O_2 and light from the lamp so that chlorella vulgaris can continue to carry out the photosynthesis process, the room temperature was set to remain constant at 28° while seawater remained constant at 27°C-28°C. After 7 days of culturing in 200-300 ml Erlenmeyer tubes, the culture was transferred to a container containing ½ liter of sterile seawater which was also given O_2, and continued in 1 and 10 liter containers respectively for 7 days with the same treatment. After 7 days of intermediate culture, water and phytoplankton were separated after 15 minutes precipitation were formed, indicating the phytoplankton and water have been separated. The precipitate was filtered and dried in direct sunlight for 3 days to form a hard gel. The gel was then blended until smooth and then filtered again to obtain a fine chlorella vulgaris powder.

Preparation of chlorella Vulgaris extract gel preparations

After the distilled water is heated, place it under the homogenizer and disperse it with a gelling agent in the form of 3 gr NaCMC, and stir until homogeneous. Add 10 g of propylene glycol, 10 g of glycerol, and 0.12 g of methylparaben, stir until homogeneous and form a gel base. Addition of 5 gr of chlorella Vulgaris extract to the gel base. Stir until the gel base and chlorella Vulgaris extract become homogeneous and form gel preparations with concentrations of 5%, 10%, and 15%.

Poliphenol screening

The sample was dissolved in a 10 mL volumetric flask using methanol as a solvent. Sample stock was put into a 5 mL volumetric

flask, and 3 replications were carried out. The sample was diluted in a 5 mL volumetric flask, reacted with 2.5 mL of 7.5% Folin ciocalteu, allowed to stand for 8 minutes and then 2 mL of 1% NaOH was added, and blue color was formed. The sample solution was allowed to stand for 60 minutes and then measured at the maximum wavelength using a Uv-Vis spectrophotometer. Standard curve of the amount of polyphenol content was obtained from the spectral measurements.

Antioxidant assay

Four mg of DPPH (diphenyl picrylhydrazyl) was dissolved in a 25 mL volumetric flask with methanol, the sample was dissolved in a 10 mL volumetric flask using methanol solvent. A stock solution of 10.000 ppm was diluted to obtain 5 different concentrations for IC50 calculation. 400, 800, 1200, 1600, 2000 ppm was placed into a 5 mL volumetric flask, and 1 mL DPPH was added to each volumetric flask. The sample was allowed to stand for 1 hour and then its absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of the DPPH.

Physical stability test of chlorella Vulgaris gel extract preparation

Spreadability test: Chlorella Vulgaris extract gel with a concentration of 5%, 10%, and 15% of 1 gram was placed on a clear glass with a thickness of 2 mm, the bottom of which was attached with millimeter paper with a diameter of 20 cm. The clear glass was covered with cloth glass which has the same area. The glass was given a load of 100 grams in which a constant diameter of distribution was obtained.

pH test: The pH of 5%, 10%, and 15%, Chlorella extract gel were measured with a standardized pH meter.

Viscosity assay: A viscosity test was carried out by placing 100 mL of 10% chlorella extract gel in a cylindrical container and then installing a spindle. The spindle must be immersed in the test preparation. The viscometer was turned on at a speed of 50 rpm. The viscometer was obseverd by pointing to the number on the viscosity scale. Viscosity test observations were carried out three times to determine whether there was a change in the viscosity of the gel preparation.

Results

The total polyphenol content in chlorella Vulgaris extract is 0.087% or equivalent to 0.87 mg of gallic acid (total polyphenols) in 1 gram of chlorella Vulgaris extract.

Absorbent	Total of polyphenol (per 100 mg sample)	Mean of polyphenol
0.060	0.084	0.087
0.063	0.087	
0.066	0.090	

Table 1. Polyphenol content of chlorella vulgaris extract.

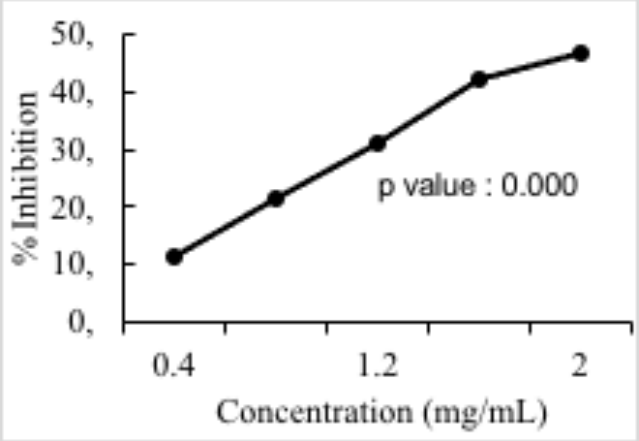

Figure 1. Antioxidant activity of chlorella vulgaris in different concentration.

Figure 1 showed the results of a high antioxidant activity test on chlorella Vulgaris extract using the DPPH method, with an IC50 value of 2 mg/mL. The data was processed using ANOVA test.

Gel concentration	Spreadability (mm^2)	Viscosity (cp)	pH
5%	154.86	12.400	9.49
10%	131.66	17.466	9.33
15%	126.73	23.066	9.17

Table 2. Spreadability of chlorella vulgaris extract gel.

From the test results, it can be seen that the gel with a concentration of 5% has the highest spreading power, with a spreading power

of 154.86 mm2 or 3.9 cm. The average test results at 50 rpm speed, the viscosity of the 5% gel are 12.400 cp, 10% is 17.466 cp and 15% is 23.066 cp. The 15% preparation has the lowest base level, namely 9.17, so it has the most satisfactory results compared to other concentrations.

Discussion

Microalgae products have been used in the health sector because these natural ingredients provide promising effects and have active ingredients needed in the wound healing process. In chlorella Vulgaris, the content of terpene derivatives and lectin compounds work as antioxidants. Antioxidant activity is also affected due to the high chlorophyll content of chlorella Vulgaris which is capable of suppressing the increase of reactive oxygen species (ROS). Unavoidable oxidative stress will activate inflammatory mediators thereby interfering with tissue regenerations[16]. In addition, other protein compounds work together in the process of forming granulation tissue, migration of keratinocytes, and stimulation of fibroblasts which will increase collagen production[17,18].

The antioxidant molecule content in chlorella Vulgaris is ascorbic acid, glutathione, vitamin E, phenolic components, and carotenoids. Microalgae extracts that contain phenol have the potential to inhibit ROS and can stimulate collagen. The high oxidative stress activity caused by ROS harms the vitality and expression of collagen in tissues. A study reported that the antioxidant effect was able to extend the lifetime of fibroblasts due to the content of vitamin E. Phenol content is also able to increase antioxidant defenses and enzyme activity, to support the function of fibroblast cells. The results of other studies reported strong antioxidant activity based on three types of solvents used to see antioxidant activity, namely non-polar, semi-polar and polar[4,19–22]. Other studies also state that there is a correlation between total phenols and antioxidant activity, in which the phenol content in plant extracts contributes to more than 50% of antioxidant activity[16]. This is in line with the phenol content found in chlorella vulgaris, so that it is able to delay or inhibit ROS and prevent tissue inflammation[23].

The spreadability test aims to determine how much the gel spreads on the skin. Another study reported that high spreading ability will determine the effectiveness of a material's biological ability when used on inflamed areas. The test results in this study showed that the spreadability of the chlorella Vulgaris gel was 3.9 cm, which indicated that the extract in the gel formulation could spread well with the characteristics of a thin layer covering the surface of the skin[17,24].

Measurement of the pH of the chlorella Vulgaris extract gel can show the level of acidity of the gel when used. A good acidity or pH level is close to neutral for the mouth area, namely 6-7, so as not to disturb the tissues in the area such as teeth and gums. The results of the pH test that had been carried out found that the chlorella Vulgaris preparation had alkaline properties. This is caused by the type of active ingredient chlorella Vulgaris which has a high base level, namely Ph 9, this is due to the presence of phenol in the extract preparation[17,19,25].

The viscosity test aims to determine the thickness of the gel preparation with a viscometer. Viscosity is a statement of the "resistance to flow" of a system under pressure. The thicker a liquid, the greater the force needed to make it flow at a certain speed. From the test results, it is known that the preparation that has a good viscosity is a gel with a concentration of 5%. This study shows the characteristics of the gel formulation is semisolid with the pseudoplastic flow, which can disperse the extract molecules to the applied area[17,26].

Conclussions

Chlorella Vulgaris extract contains phenolic compounds which show antioxidant activity and the gel preparations from chlorella Vulgaris extract also have the basic characteristics needed in the formation of new tissues, including good physical stability, biological activity, and other biological functions.

Acknowledgments

The author would like to thank the Balai Budidaya Air Payau Takalar, Biopharmaca Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar for supporting this research

and thank to Kemendikbud Ristek Indonesia for funding this research through PDD Grant with contract number 091/E5/PG.02.00.PT/2022.

Declaration of Interest

The authors report no conflict of interest.

References

1. Hamdy O, Karim A, Farouk Gheda S, Ismail GA, Abo-Shady AM, Assistant Of Phycology T. Delta Journal of Science Phytochemical Screening and antioxidant activity of Chlorella vulgaris. Djs. 2020;41:81–91.
2. Anwer SS, Sdiq KH, Muhammad KR, Aladdin LM. Phenolic compound and fatty acid properties of some microalgae species isolated from Erbil City. Brazilian J Biol. 2022;82: 1-8.
3. Syed S, Arasu A, Ponnuswamy I. The uses of Chlorella Vulgaris as antimicrobial agent and as a diet: The presence of bio-active compounds which caters the vitamins, minerals in general. Int J Bio-Science Bio-Technology. 2015;7(1):185–90.
4. Coulombier N, Jauffrais T, Lebouvier N. Antioxidant compounds from microalgae: A review. Mar Drugs. 2021;19(10). 1-30.
5. Park YJ, Park J-E, Truong TQ, Koo SY, Choi J-H, Kim SM. Effect of Chlorella vulgaris on the Growth and Phytochemical Contents of “Red Russian” Kale (Brassica napus var. Pabularia). Agronomy. 2022;12(9):2138.
6. Coronado-Reyes JA, Salazar-Torres JA, Juarez-Campos B, Gonzalez-Hernandez JC. Chlorella vulgaris, a microalgae important to be used in Biotechnology: a review. Food Sci Technol. 2020;42: 1-9.
7. Zailan N, Abdul Rashid AH, Das S, Abdul Mokti NA, Hassan Basri J, Teoh SL, et al. Comparison of Chlorella vulgaris dressing and sodium alginate dressing: an experimental study in rats. Clin Ter. 2010;161(6):515.
8. Edwin S, Jarald EE, Deb L, Jain A, Kinger H, Dutt KR, et al. Wound healing and antioxidant activity of achyranthes aspera. Pharm Biol. 2008;46(12):824–8.
9. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010;2(12):1231–46.
10. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165–70.
11. Martins N, Barros L, Ferreira ICFR. In vivo antioxidant activity of phenolic compounds: Facts and gaps. Trends Food Sci Technol. 2016;48:1–12.
12. Fernandez-Panchon MS, Villano D, Troncoso AM, Garcia-Parrilla MC. Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. Crit Rev Food Sci Nutr. 2008;48(7):649–71.
13. Süntar I, Akkol EK, Nahar L, Sarker SD. Wound healing and antioxidant properties: do they coexist in plants?. Free Radicals Antioxidants. 2012;2(2):1–7.
14. Hasan H, Machmud E, Alpiyanti P. The effect of Chlorella vulgaris salep extract on the formation of bone remodeling. Makassar Dent J. 2020;9(3):170–3.
15. Schmid GJ, Kobayashi C, Sandell LJ, Ornitz DM. Fibroblast growth factor expression during skeletal fracture healing in mice. Dev Dyn an Off Publ Am Assoc Anat. 2009;238(3):766–74.
16. Suwannakul S, Chaibenjawong P, Suwannakul S. Antioxidant Anti-Cancer and Antimicrobial Activities of Ethanol Pandanus amaryllifolius Roxb. leaf extract (In Vitro)-A potential medical application. JIDMR. 2018;11(2): 383-88.
17. Andrade AF, Egito MS, Vilela WT, Costa R, Rolim-Neto PJ, Silva RMF, et al. Wound healing gel based on Chlorella vulgaris extract characterization. Biointerface Res Appl Chem. 2021;12:4463–75.
18. Agustina S, Aidha NN, Oktarina E. The extraction of antioxidants from Chlorella vulgaris for cosmetics. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing; 2021:12057.
19. Kurnia D, Rosliana E, Juanda D, Nurochman Z. Antioxidant Activity and Determination of Total Phenol Content of Marine Microalgae Chlorella vulgaris. J Kim Ris. 2020;5(1):14–2.
20. Merecz-Sadowska A, Sitarek P, Kucharska E, Kowalczyk T, Zajdel K, Cegliński T, et al. Antioxidant properties of plant-derived phenolic compounds and their effect on skin fibroblast cells. Antioxidants. 2021;10(5):726.
21. Sadowska-Bartosz I, Bartosz G. Effect of antioxidants on the fibroblast replicative lifespan in vitro. Oxid Med Cell Longev. 2020;2020(9):1-6.
22. Cao X-P, Chen Y-F, Zhang J-L, You M-M, Wang K, Hu F-L. Mechanisms underlying the wound healing potential of propolis based on its in vitro antioxidant activity. Phytomedicine. 2017;34(10):76–84.
23. Ma’ruf MT, Dewi PS, Nurlitasari DF. Efficacy of Bidara Leaf (Ziziphus Mauritiana) Viscous Extract to Gingival Wound Healing in Wistar Rats. JIDMR. 2021;14(4):1367–72.
24. Prasetya F. Characteristics and Stability of Mouth Gel with Active Ingredients of Black Betel Leaf Extract Based on Hydroxy Ethyl Cellulose (HEC). J Trop Pharm Chem. 2013;2(2):83–90.
25. Tunjungsari D. Formulation of Ethanolic Extract Gel of Mahkota Dewa Fruit (Phaleria macrocarpa (Scheff) Boerl.) with Carbomer Base. Muhammadiyah University Surakarta. 2012.
26. Dantas MGB, Reis SAGB, Damasceno CMD, Rolim LA, Rolim-Neto PJ, Carvalho FO, et al. Development and evaluation of stability of a gel formulation containing the monoterpene borneol. Sci World J. 2016;2016(4): 1-4.