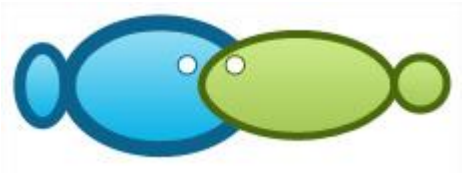

Antioxidant and proximate analysis on agar from *Eucheuma denticulatum* algae, supplemented with chlorophyll pigments extracted from pandan leaves *Pandanus amaryllifolius*

Desy M. H. Mantiri, Rene C. Kepel, James J. H. Paulus, Nickson J. Kawung, Darus S. J. Paransa, Marselino S. Gerung

Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado, North Sulawesi, Indonesia. Corresponding author: D. M. H. Mantiri, dmh_mantiri@unsrat.ac.id

Abstract. The aim of this research was to create an agar from the red algae *Eucheuma denticulatum* cultivated in the waters of Nain Island, Wori District, North Minahasa Regency, North Sulawesi Province. Agar is prepared by adding chlorophyll pigment from pandan leaves, *Pandanus amaryllifolius*. The process of making agar consisted of mixing young coconut water and chlorophyll with several concentrations using a Completely Randomized Design, then a toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method. Proximate analysis was conducted to determine the quality of agar material as food. Antioxidant test was carried out using the 1,1-diphenyl-2picrylhydrazil method and fungal growth analysis was carried out on Plate Count Agar (PCA) solid media. The results obtained showed a good green color change on the agar with a gel strength of 314.0676 g cm^{-2}. The toxicity test showed that the percentage of mortality was 15% at a concentration of 5.0 ppm. The antioxidant test showed that with the addition of chlorophyll pigment, the agar had antioxidant activity with an IC_{50} value of 83.4167 ppm which was categorized as strong. Proximate analysis showed a water content of 24.85%, an ash content of 1.1%, a fat content of 0.73, a protein content of 1.04%, a carbohydrate content of 27.54%, and a crude fiber content of 4.94%. Meanwhile, the fungal test showed that fungal started to grow on the fourth day as much as 10 CFU g^{-1} (control) and 27 CFU g^{-1} (Chlorophyll).
Key Words: morphological identification, gel strength, ash content, fungal contamination, toxicity test.

Introduction. Marine macroalgae have long been known as additional food ingredients, vegetables and traditional medicines, and, through the progression in technology, various active compounds for medicines, cosmetics and nutraceutics were discovered. Marine algae produce colloidal compounds known as phycocolloids namely agar, algin and carrageenan. Red algae (Rhodophyta) contain pigments such as phycoerythrin, chlorophyll a and chlorophyll d, carotene, and xanthophyll (Mantiri et al 2021a; Kasanah et al 2015). Several researches showed that in crude extracts fractionated parts and/or pure compounds from green, brown and red marine algae have antioxidant (Mantiri et al 2021b; Corsetto et al 2020), anticoagulant (Wijesinghe et al 2011), antiviral (Cheung et al 2014), antifungal (De Corato et al 2017), anticancer (Gutiérrez-Rodríguez et al 2018), cytotoxic (Gunasekaran et al 2017), anti-inflammatory (Kazłowska et al 2010), and antibacterial (Agbaje-Daniels et al 2017) activity.

Compared to other algae, red algae possess more biological activity. This alga is the main source for formation of halogenated compounds. *Eucheuma denticulatum* is one of red algae species that contains many important elements such as calcium, copper, iron, iodine and nitrogen. This species also contains chlorophyll a and chlorophyll d pigments, carotene, lutein, and phycoerythrin. In addition, it contains protein, carbohydrates, fat, water, fiber and ash (Trono 1997). The aim of this research was to create agar from *E. denticulatum* with the addition of chlorophyll pigment from pandan leaves *Pandanus amaryllifolius*.

Material and Method

Study area. Samples of *E. denticulatum* were collected from an algae cultivation area in Nain Island, North Minahasa Regency, North Sulawesi Province (Figure 1). Algae was identified morphologically by comparing the blade, stipe and holdfast characteristics referring to the algae identification book of Trono (1997). This examination was carried out at the Laboratory of Marine Biology, Faculty of Fisheries and Marine Sciences (FPIK), Sam Ratulangi University (UNSRAT).

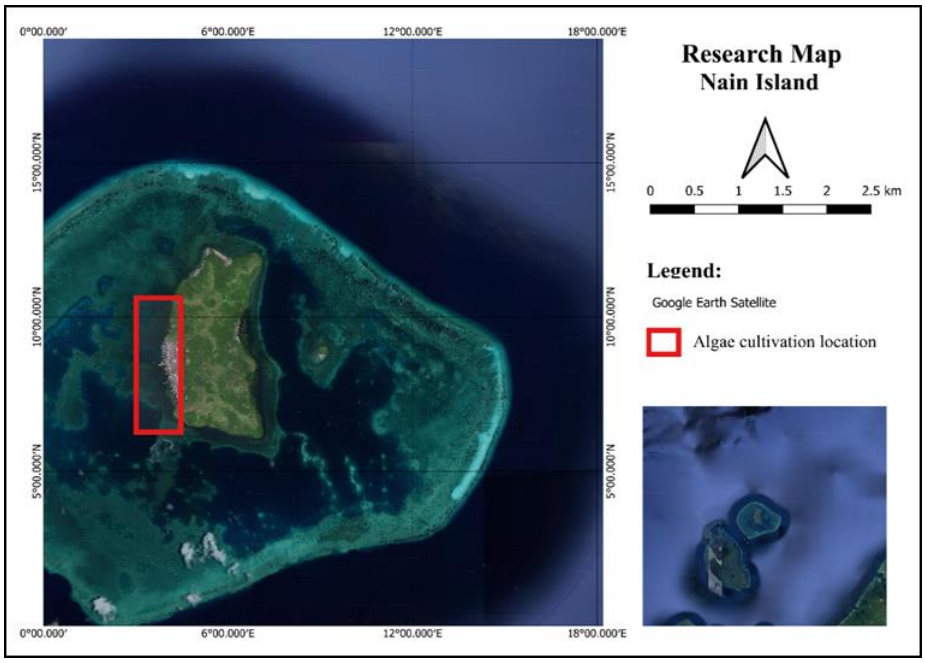

Figure 1. Map of the study area.

Chlorophyl pigment extraction. Extraction of chlorophyll pigment from *P. amayllifolius* leaves was carried out following the method of Kurniawan et al (2022). The procedures were: 100 g of pandan leaves were weighed and cut into 2 cm pieces. The cutting was then put into a blender, 500 mL of water was added, then the mixture was ground until smooth and then filtered using a gauze. This extraction was carried out at the Laboratory of Molecular Biology and Marine Pharmacology, FPIK, UNSRAT.

Production of agar with the addition of chlorophyll pigment. *E. denticulatum* sample was air-dried, then weighed to obtain as long as much as 100 g, washed and soaked in 0.15% calcium hypochlorite $Ca(ClO)_2$ solution for 30 minutes until the algae color became pale white. Algae were then washed with running water repeatedly until it had no smell, then lime juice of *Citrus aurantifolia* was added before processing. The initial stage of agar production was 100 g of algae was cooked in 600 mL of coconut water for 30 minutes at a temperature of 80-90^{o}C. After the algal thallus had dissolved in coconut water, 75-100 mL of chlorophyll pigment were added. The solution was then filtered, printed and cooled. This research was conducted at the Laboratory of Molecular Biology and Marine Pharmacology, FPIK, UNSRAT.

Toxicity test method. The toxicity test of agar previously added with chlorophyll pigment was carried out using Brine Shrimp Lethality Test (BSLT) method using *Artemia salina*. Observations were conducted for 24 hours to observe the number of test animals which died.

Antioxidant test method. Antioxidant activity tests on chlorophyll pigment-added agar and control (agar without dye) were conducted using 0.1 mM DPPH Radical (1,1-diphenyl-2-picrylhydrazyl). DPPH powder was dissolved in pro-analysis (p.a.) methanol.

The maximum wavelength of DPPH was 517 nm. Blank solution of 2 mL of 0.15 mM DPPH was put into a test tube, added with 2 mL of methanol, stirred until homogeneous, and then incubated in a dark room for 30 minutes. Afterwards, the absorption was measured with a UV-Vis Spectrophotometer at a wavelength of 517 nm. As much as 50 mg of chlorophyll-added agar and 50 mg of control samples were each dissolved in p.a. methanol, then put into a 10 mL flask. The flask was filled adequately with p.a. methanol to the boundary sign. Each sample was prepared in methanol concentrations of 50, 100, 150, 200 and 250 ppm. Free radical catching activity can be expressed with IC_{50} (inhibitory concentration value). IC_{50} is the concentration of the test compound resulting in 50% loss of free radical activity. The smaller the IC_{50} value, the higher the antioxidant activity. Radical antidote activity is expressed as inhibition percentage which can be calculated by the following formula of Meyer et al (1982).

Proximate test method

Water content analysis. The AOAC (2005) method was applied: an empty Petry disk was dried in an oven at 105^0C for 1 hour. Afterward, then cooled in a desiccator for 20-30 minutes and weighed to obtain its empty weight (constant weight). Next, the sample was weighed to obtain 1 g, then placed in the empty disk and all was placed in the oven at 105^0C for 8 hours. The sample that no longer contained water was taken and placed in a desiccator for 20-30 minutes, then weighed. The percentage of water content is calculated using the formula (Sudarmadji et al 2010):

$$\text{Water content (\% wm)} = \frac{A-(C-B)}{A} \text{ x } 100\%$$

Where:
A - sample wet weight/before being dried in the oven (g);
B - dry disk weight/constant (g);
C - dry disk + dry sample weight (g).

Ash content analysis. The AOAC (2005) method was applied: an empty porcelain disk was dried in a furnace for 1 hour then cooled in a desiccator for around 1 hour until cold. The empty porcelain disk was then weighed and its weight was recorded. The sample was weighed; about 3 g were retained and put in the disk, then burned on an electric stove until the sample did not smoke. Ashing was done in furnace at a temperature of 550°C for 3-4 hours or until the sample completely became white ash. Subsequently, the porcelain disk was cooled in a desiccator until cold. Then the disk contained the sample was weighed and recorded. The percentage of ash content can be calculated by the following formula (Sudarmadji et al 2010):

$$\text{Ash content (\%)} = \frac{\text{Ash weight}}{\text{Sample weight}} \text{ x } 100$$

Fat content analysis. The soxhlet method (AOAC 2005) was applied: 1-2 g of fresh sample was weighted and put on a paper covered with cotton. The fresh sample was dried first using a rotary evaporator before being weighed and put into cotton. The paper containing the sample was plugged with cotton, dried in an oven for about 1 hour at a temperature of no more than 80ºC, inserted into a soxhlet which had been connected to a fat flask containing dried boiling stones in which its weight was already determined. After that, sample was extracted using hexane or other fat solvent for around 6 hours. Hexane was then distilled and the extract was dried in an oven at 105ºC. The fat extract obtained was cooled and weighed. Drying was done repeatedly until a constant weight was achieved. The fat content was calculated as follows (Sudarmadji et al 2010):

$$\text{Fat content (\%)} = \frac{w-w1}{w2} \text{x } 100$$

Where:
w - sample weight (g);
w1 - fat weight before extraction (g);
w2 - fat flask weight after extraction (g).

Protein content analysis. Protein content was analyzed by putting 1.5 g of sample into a scale tube, then added 7.5 mL of distilled water. The mixture was homogenized using a vortex, centrifuged for 15 minutes, then separated the supernatant from the precipitate. The supernatant was boiled on a hotplate. The sample was centrifuged for 15 minutes and then it was separated from its supernatant for analyzing. Next, 2 mL of the supernatant were taken, added with 1 mL of 10% TCA solution, then centrifuged for 15 minutes to separate the solution and precipitate. As much as 0.1 mL of TCA sample extract was added with 1.9 mL of distilled water and 2.5 mL of Lowry's reagent. Furthermore, the mixture was homogenized and stored at room temperature for 10 minutes. Furthermore, the solution was added with 0.5 mL of Folin's reagent and incubated at room temperature for 30 minutes until a blue color formed. Next, the absorbance of sample was measured on a spectrophotometer at a wavelength of 600 nm, using a standard Bovine Seru Albumin (BSA) solution. The protein content could be calculated using a regression equation.

Crude fiber analysis. A total of 5 g of sample was placed in an oven-dry porcelain disk, added with 50 ml of 1.25% sulfuric acid, and stored in a beaker glass right under the condenser (reflux), then put under water and the solution was boiled in the electric heater for 30 minutes from the time it started to boil. Afterward, as much as 100 ml of 1.25% NaOH was added, then the beaker glass was placed on the special heating device. After adequate heating, solution was filtered using a Buchner funnel previously fitted with a filter paper with a known weight. The filter paper was washed/rinsed successively with: hot water 100 mL, runny sulfuric acid 0.3 N (1.25%) 50 mL, hot water 100 mL and acetone 50 mL. Next, the filter paper and its residue were put into a porcelain disk using tweezers, dried in an oven at 100-105ºC for 1 hour, cooled in a desiccator for 15 minutes and then weighed. Crude fiber was determined as follows (Sudarmadji et al 2010):

$$\text{Crude fiber (\%)} = \frac{C-B}{A} \text{x } 100$$

Where:
A - sample weight;
B - constant filter paper weight;
C - weight of filter paper + residue.

Carbohydrate content analysis. The AOAC method (2005) was applied: the carbohydrate content was determined "by difference" method, where the final value of the percentage of carbohydrates in agar (from 100%) must be reduced by the content of water. Carbohydrate levels can be calculated by the formula (Sudarmadji et al 2010):

$$\text{Carbohydrate content (\%)} = 100 - A - B - C - D$$

Where:
A - water content (%ww);
B - ash content (%ww);
C - protein content (%ww);
D - fat content (%ww).

As much as 1 g of sample was placed in an Erlenmeyer and added with 100 mL of 3% HCl. The solution was hydrolyzed for 2.5 hours by placing it in an upright cooler. The solution was then cooled and neutralized with 25 mL of 4N NaOH. Afterward the solution was placed into a 250 mL volumetric flask and made up with distilled water to the mark sign. The solution was filtered with filter paper and hold in a beaker glass. As much as 10 mL of solution was pipetted and put in a 250 mL Erlenmeyer, then 25 mL of luff solution and several boiling stones were added and then boiled for 10 minutes under the upright cooler.

The solution was subsequently added with 20 mL KI 20% and 25 mL H2SO4 25%. The solution was then titrated with 0.1 N Na-thiosulfate (which had been standardized). The titration was terminated when the solution changed to an ivory yellow color. The solution was then added with starch indicator until the solution turned blue and the

titration was continued until the solution color became milky white. An amount of 2.0 mL of prepared sample solution was pipetted into a 25.0 mL volumetric flask then added with distilled water up to the mark. 0.5 mL of the solution were pipetted into a test tube, added with 5.0 mL of anthrone reagent, then covered and shaked until evenly distributed. After mixing evenly, the solution was heated in a 100°C water bath for 12 minutes. The solution was cooled for 1 minute, then the absorbance at a maximum wavelength of 630 nm was determined. Data of absorbance and samples were then entered into a linear regression equation between concentration and absorbance. The r value close to ±1 indicate a linear curve. A high r suggests a very strong correlation between two variables, namely variable X as concentration and variable Y as absorbance (Riyanto 2011). The glucose content was calculated by the formula:

$$y = bx + a$$

Where:
y - absorbance;
x - content;
a - constant;
b - regression coefficient.

Glucose quantitative

Preparation of glucose stock standard solution 1000 ppm. As much as 100 mg of standard glucose was weighted, put it in a 100.0 mL volumetric flask and diluted with distilled water up to the mark.

Preparation of 0.1% anthrone reagent. Anthrone reagent 0.1% was prepared by dissolving 100 mg of anthrone in concentrated sulfuric acid until the volume reached 100.0 mL. A total of 1 mL standard glucose working solution of 150 ppm was pipetted, then added with 5 mL of anthrone reagent, closed in the tube and shaked to mix thoroughly. The solution was then heated in a water bath at 100°C for 12 minutes, left for 1 minute, then the absorbance at the wavelength range of 610-640 nm was determined.

Preparation of standard basic curves (50, 75, 100, 125, 150, and 175 ppm). A pipette of 0.5 mL was used to take samples from 0.75; 1; 1.25; 1.5; 1.75 mL of 1000 ppm glucose stock solutions, then to put them into a 10.0 mL volumetric flask and add with distilled water up to the mark. The solution was transferred into a test tube, added 5.0 mL of anthrone reagent into each test tube, closed and shaken to mix thoroughly. Afterward, the solution was heated it in a 100°C water bath for 12 minutes, cooled for 1 minute, then the absorbance at a maximum wavelength of 629.1 nm was determined. Measurements of absorption of the standard solutions (of the serial dilution) were carried out at maximum wavelengths started from the smallest levels. The linear regression equation, which is the relationship between concentration and absorbance, was calculated and the correlation coefficient was determined. A calibration curve between concentration and absorbance was then created.

Samples determination. A total of 2.0 mL of prepared sample solution was pipetted into a 25.0 mL volumetric flask then added with distilled water up to the mark. As much as 0.5 mL of solution was pipetted into a test tube, added with 5.0 mL of anthrone reagent, then covered and shaked until evenly distributed. After mixing evenly, the solution was heated in a 100°C water bath for 12 minutes. Solution was cooled for 1 minute, then the absorbance at a maximum wavelength of 630 nm was determined. Data of absorbance and samples were then entered into a linear regression equation between concentration and absorbance.

Fungal test

Preparation of agar media. Solid media of Plate Count Agar (PCA) was prepared by weighing 11.75 g of PCA powder, dissolved in 500 mL of distilled water in an Erlenmeyer

flask then heated until boiling. The media was then sterilized in an autoclave for 15 minutes at a temperature of 121°C and a pressure of 15 psi (per square inch) (Volk & Wheeler 1990).

Treatment in laminar air flow. Prepare 10 test tubes each containing 4 mL of sterile distilled water. Active culture solution about 1 mL was taken with a micro pipette then placed into the first tube then homogenized to obtain a dilution concentration of 10^{-1}. A concentration of 10^{-2} was made by taken 1 mL of solution from the first tube then put it in the second tube and homogenized. A concentration of 10^{-3} was prepared by taking 1 mL of solution from the second tube, put it in the third tube and then homogenize, and so on until obtained the fourth dilution. 1 mL of solution was taken with a micro pipette from each of the tubes and then put into a Petri dish. Furthermore, 10 mL of liquefied solid media was added. The solution in each Petri dish was shaken so that the solid media was evenly distributed, then it was left for a few minutes to solidify. The Petri dish was subsequently kept upside down in an incubator at 37°C for 24 hours. The number of colonies grown in the media was counted using the Colony Counter tool.

Results and Discussion

Morphological identification. *E. denticulatum* algae has a round-upright thallus, 5-30 cm long, transparent, yellowish brown to yellowish red. The surface of the thallus is covered with bulge shaped like irregular pointed spines. Some of these spines are elongated, shaped like branches. The algae have dense branches and can form bunches. The thallus' branches grow on the old and young parts irregularly. *E. denticulatum* grows attached to coral reefs, corals, rocks, hard objects, and shell of shellfish. Sequencing and morphological analysis of this red algae species used in this study identified as *E. denticulatum* with a confidence level of 99.53% (Tumembouw 2022) (Figure 2).

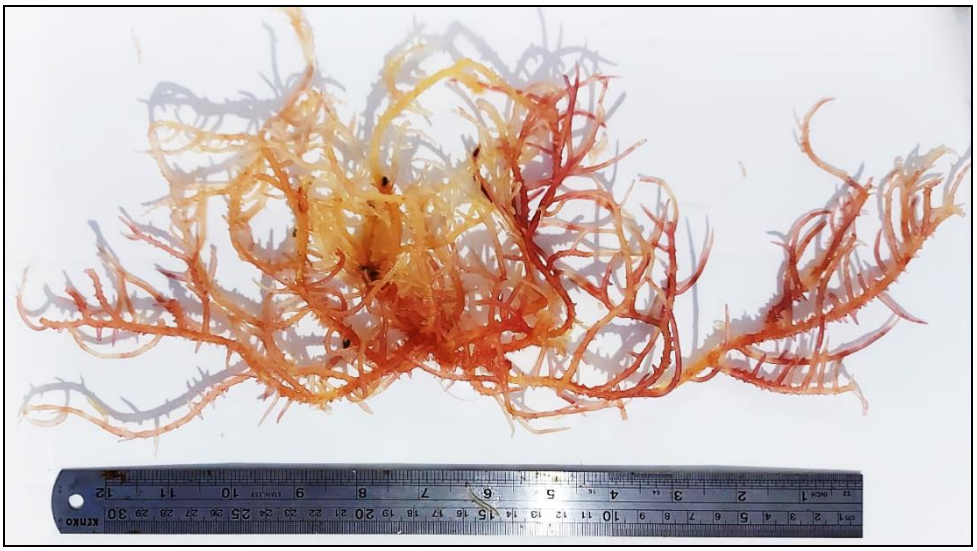

Figure 2. *Eucheuma denticulatum.*

Chlorophyll pigment extraction. Preparation of chlorophyll pigment extract from pandan leaves referred to Kurniawan et al (2022). The preparation stages were as follows: 100 g of pandan leaves were weighed, washed and cut into 2 cm pieces, added with 500 mL of water, then smoothed with a blender, filtered and placed in a beaker glass (Figure 3).

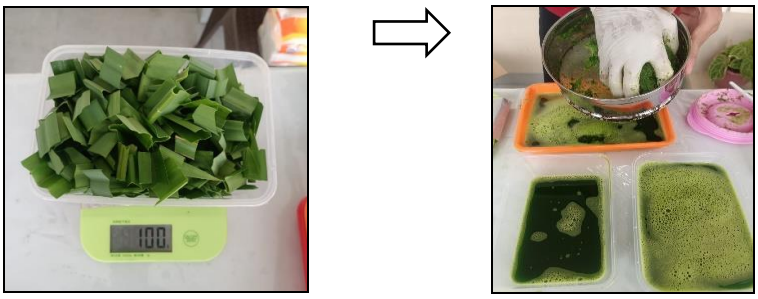

Figure 3. Preparation of chlorophyll pigment.

Agar preparation from E. denticulatum with the addition of chlorophyll pigment. Agar obtained from a boiling process with coconut water added with chlorophyll pigment had a gel strength of 314.0676 g cm^{-2}, while the strength control gel was 579.1896 g cm^{-2}. The strength of control agar was stronger than that of agar with the addition of chlorophyll pigment. This was due to the addition of chlorophyll pigment resulting in a decrease in gel strength (Figure 4).

Figure 4. Preparation of agar from algae with the addition of chlorophyll pigment.

The agar gel strength indeed depends on the ratio of agarose to agaropectin content. The gel formed will be stronger with the increase of agarose content. Gel formation is a phenomenon of combining or cross-linking polymer chains that form a continuous three-dimensional mesh. Furthermore, this gel binds or immobilizes the water in it and forms a strong and rigid structure. The characteristics of this gel formation vary from one of another hydrocolloids, depending on the hydrocolloid type. Gels had solid-like characteristic especially elastic and stiffness (Fardiaz 1989). The gel strength of agar can provide a flavorful texture on food.

Antioxidant analysis

Antioxidant activity of agar. Measurement of the antioxidant activity of agar was carried out using a UV-Visible spectrophotometer with a wavelength obtained of 517 nm. Antioxidant activities obtained were shown in the following Table 1.

Table 1

Antioxidant activity of agar

Samples	*Concentration (ppm)*	*Barrier activity*	*IC_{50} (ppm)*
Control	50	39.92	124.0395
	100	47.89	
	150	53.10	
	200	60.08	
	250	65.46	
	Control DPPH	0	
Agar + chlorophyll pigment	50	46.28	83.4167
	100	51.29	
	150	59.18	
	200	61.89	
	250	69.96	
	Control DPPH	0	

It was found that the IC_{50} value of the agar sample with the addition of chlorophyll pigment had an antioxidant strength level of 83.4167 ppm, which was categorized as strong antioxidant activity, while antioxidant strength of agar without addition of pigment was 124.0395 ppm, categorized as medium. It can be concluded that agar with the addition of pigment has stronger antioxidant activity than that of agar without addition of pigment. Agar is categorized as having strong antioxidant activity if it has an IC_{50} value of 50-100, while medium activity has an IC_{50} value of 100-150. Agar has the potential as an antioxidant because it has a significant number of hydroxyl groups which are able to donate protons to DPPH free radicals.

Toxicity test. Toxicity test is a screening method to determine the toxicity of a natural extract or compound. The Brine Shrimp Lethality Test (BSLT) method is used for screening natural materials. The BSLT method is an acute toxicity test where the toxic effect of a compound is determined within a short time, that is 24 hours after administration of the test dose. A chemical capable of killing is declared to be toxic (Meyer et al 1982). An extract is declared very toxic for an LC_{50} (Lethal Concentration 50%) value below 30 ppm, toxic for an LC_{50} value between 30 - 1000 ppm, and non-toxic for an LC_{50} value above 1,000 ppm (Figure 5).

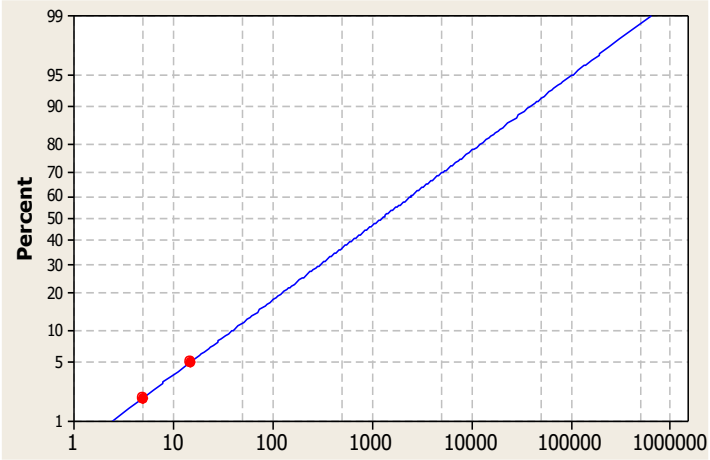

Figure 5. Graph of probit analysis results.

Research result showed that chlorophyll pigment from *E. denticulatum* had an LC_{50} of 1,245 mg mL^{-1}, considered as nontoxic (Meyer et al 1982). This proves that this pigment is very safe for use in food and as a natural dye. Anderson (1991) reported that an extract showed toxic activity in BLST if the extract could cause 50% mortality of the test animals at a concentration of less than 1,000 ppm.

Proximate analysis. Proximate analysis results from *E. denticulatum* agar with the addition of chlorophyll pigment from pandan leaves were as follows:

Water content. Is a characteristic greatly influences food ingredients, especially on the appearance, texture and taste of food. High water content causes bacteria, mold and yeast to grow easily, resulting in changes to food ingredients (Winarno 1991). Water content is one of the considerations in food processing and greatly influences the quality of food ingredients. Water is often reduced by evaporation, thickening and drying. High water content in food will enable changes in chemical and biochemical, as well as the growth of microorganisms during the storage period (Hall 1980). The water content required by the quality standard SNI 2802:2015 (SNI 2015) is 22%, whereas in this research, the water content of agar with the addition of chlorophyll pigment was 24.85%, exceeding the established quality standards.

Ash content. Is an inorganic mineral that does not evaporate easily and it is the residue left after the sample is burned and ignited till free of carbon and water. The ash content

contained in a product indicates the purity level of that product. The purity level of a product is very influenced by the composition and its mineral content (Darmawan et al 2006). The ash content, as required by SNI 2802:2015 (SNI 2015) is of maximum 6.5%, whereas in this study the ash content obtained was 1.1%.

Fat content. Fat and oil are very important elements for maintaining the health of the human body. The quantity of fat component in agar is small and it is used as a complement to the agar. In this research, the fat content of the *E. denticulatum* agar was 0.73%.

Protein content. Is an important food substance for the body because it functions as a source of energy, building blocks and regulating substances (Winarno 1991). The protein component in agar is present in small quantities and is a complementary component to agar. The protein content of *E. denticulatum* agar in this study was 1.04%.

Crude fiber. Food fiber is the part of food that is resistant to the hydrolysis process by digestive enzymes in stomach and small intestine (Kusharto 2006). Crude fiber, which is commonly used in proximate analysis of food ingredients, is part of food fiber that cannot be hydrolyzed by H_2SO_4 and NaOH during the process of determining crude fiber. In moderate amounts, crude fiber is required to simplify the work of the digestive system, absorption of various nutrients, and provide taste of food. The research results by the Japanese Ministry of Health showed that agar is a high fiber food, so it is recommended to be consumed to prevent colon cancer, hemorrhoids and obesity (Anggadireja et al 2006). The crude fiber content of *E. denticulatum* agar in this research was 4.94%, showing a greater value as compared to the results of Susanto et al (1978), namely 0.1%. It was suspected that during the hydrolysis process in strong acids and dilute strong bases, protein was also hydrolyzed, so fiber content increased.

Carbohydrate levels. Carbohydrate levels have an important role in determining the characteristics of food ingredients, such as taste, color and texture (Winarno 1991). In general, carbohydrates found in nature occur as polysaccharides with high molecular weight. Galactose is the monomer of the largest polysaccharide found in agar. The carbohydrates contained in agar are a mixture of polysaccharides consisting of two fractions, agarose and agaropectin. The carbohydrate content found in this study was 27.54%.

Total viable count. Total viable count (TVC) is a number that indicates the number of colonies of mesophilic aerobic bacteria present per milliliter of test sample (Yusmaniar et al 2017). Using plate count agar media on agar from the algae *E. denticulatum* with an addition of chlorophyll on the first day of storage was 47 CFU g^{-1}, on the second day was 24 CFU g^{-1}, on the third day 37 CFU g^{-1} while agar without addition of chlorophyll on the first day 21 CFU g^{-1}, second day 19 CFU g^{-1}, and third day 180 CFU g^{-1}. Referring to SNI 2802:2015 (SNI 2015), the maximum TVC contamination is 5000 CFU g^{-1}.

Fungal test. Mold/yeast testing is a mandatory parameter for every food product. The maximum requirement for food product is 300 CFU g^{-1} as established by SNI 2802:2015 (SNI 2015). In this study, no fungal growth was found that on day 1 to day 3. Fungi started to grow from day 4 to day 7. On day 7, the growth of fungi increased. In control, the average colony on day 4 was 10 CFU g^{-1} and day 7 was 36 CFU g^{-1}, in agar added with chlorophyll pigment average colony on day 4 was 27 CFU g^{-1} and on day 7 it was 33 CFU g^{-1}. It can be concluded that on the fourth day of storage there was a tolerable fungal growth until the fifth day, still within the quality standard, namely 300 CFU g^{-1}.

Conclusions. The results show that the agar from the algae *E. spinosum* succeeded in having a good color with the addition of chlorophyll from pandan leaves *P. amaryllifolius*. This agar product has a very good quality composition, in accordance with Indonesian

national standards, has strong antioxidant activity and is not toxic, so it is safe for consumption.

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Conflict of interests. The authors declare no conflict of interest.

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Authors:
Desy Maria Helena Mantiri, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: dmh_mantiri@unsrat.ac.id
Rene Charles Kepel, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: renecharleskepel65@gmail.com
James Jobert Hanoch Paulus, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: jamespaulus@unsrat.ac.id
Nickson Jaghson Kawung, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: kawung.nick@gmail.com
Darus Saadah Johanis Paransa, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: darusparansa@yahoo.com
Marselino Stevano Gerung, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia, e-mail: marselino_gerung@yahoo.com
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