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Antioxidant Activity and Antibacterial Seaweed Methanol Extract (*Sargassum Duplicatum J. Agardh*) and Its Potential as a Natural Preservative Alternative to Salted Eggs

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Abstracts

The research activity of the antioxidant and antibacterial esktrak methanol seaweed Sargussum duplicatum J. Agardh as well as its potential as an alternative natural preservative salted egg has been done. Seaweed extract S. duplicatum Jj. Agardh assayed phytochemistry and the content of total phenolic with variation of temperature. Metabolite secondary in seawed extract obtained through maseration using methanol solvent. The extract are partitioned, R_1 and R_2. R_1 sample is a sample without treatment and sample R_2 is a sample with 45 minutes for warming treatment in waterbath temperature to 100 °C. Phytochemical content in seawed of *S. duplicatum* J. Agardh are flavonoid dan steroid. Phytochemical content and phenolic total have positive correlation to antioxidant activity. Antioxidant activity with DPPH method yielded IC50 = 143.03 µg/mL (R_1) and 357.95 µg/mL (R_2). Antibacterial activity assay carried out trough diffusion method with SSA media (*Salmonella-Shigella* Agar). Inhibition value toward Salmonella is 1.120 mm and 1.15 mm with control is chloramphenicol. *Salmonella sp.*is pathogenic bacteria that presents in egg and causes decay on the egg. Discussion about antibacteria activity results of seawed extract of *S. duplicatum* J. Agardh are suggested to apply toward egg preservation method.

Key words: Antibacteria; Activity; Antioxidant; Salted Egg; *Salmonella sp.*

1. Introduction

Salted eggs is eggs preserved by marinated. Egg salting process aims to remove the fishy and create a distinctive flavor and also to prolong egg shelf life. Salting is the process of penetrating salt into salted material by diffusion after the ionized salt becomes Na^+

and Cl^-. Salt serves as a preservative that prevents the decay of eggs, thereby increasing the storage power. Salt content in eggs can inhibit the development of microorganisms in the egg, so the eggs can be stored in a relatively long time. Damage to the eggs can occur because the eggs are stored in open space for several weeks without preserving or cracking occurs in the egg shell that causes microbes to easily enter into eggs so that microorganisms can grow. High nutrient content in eggs is a good medium for the growth and development of microorganisms, both microorganisms that cause damage to eggs and microorganisms that cause health problems in humans who consume these eggs. Microbes will contaminate the eggshells that enter through the pores of the egg and the egg membrane to become rotten, and produce toxins that can cause food poisoning. Salmonellosis is one type of toxin or a disease caused by Salmonella. This disease can occur as a result of consuming Salmonella contaminated food/water. According to Humphrey, salmonella can penetrate the inside of the egg through the pores or cracks in the eggshell [1].

The quality of the products derived from livestock should be free of pathogenic bacteria including Salmonella sp. The quality of a food can be seen from the existence of microorganisms [2]. Methods of preservation to maintain the quality of salted eggs with salted egg storage in cold temperatures. This cooling method is not much done by producers, due to increase production costs so that economically less profitable. Alternative solutions can use natural preservatives that are safer for consumption. Natural ingredients that contain antimicrobial compounds and are easy to find are seaweed [3].

Seaweed, especially Phaeophyceae (Sargassum) is widespread in tropical waters, including Indonesia [4]. The species of Sargassum sp. Known in Indonesia there are about 12 species, one of which is (Sargassum duplicatum J. Agardh). S. duplicatum J. Agardh is a seaweed that has not been cultivated, because the acquisition of this type of seaweed is still wild in nature. Scientific research proves that S. duplicatum seaweed extract J. Agardh contains bioactive compounds such as flavonoids, saponins, terpenoids, and tannins [5] This phenol compound is one of the plant bioactive compounds that are as antimicrobial [6]. This compound will cause damage to the bacterial cell wall. This bacterial cell membrane destruction begins with the H + ions of the phenol compounds and their derivatives which will attack the polar group (phosphate group) so that the phospholipid molecules will break down into glycerol, carboxylic acid, and phosphoric acid. This results in phospholipids unable to maintain the cell membrane form, so the membrane will leak and the bacteria will be inhibited even death [7]. This phenol compound is one of the plant bioactive compounds that can also be as an antioxidant [8]. Antioxidants are compounds that can inhibit oxidation reactions or a substance that can neutralize free radicals. This compound is also very beneficial for health and plays an important role to maintain the quality of food products [9].

The content of phenolic compounds in seaweed S. duplicatum J. Agardh able to inhibit the growth of bacteria so that seaweed S. duplicatum J. Agardh has potential as a natural food preservative. Therefore, research on the antibacterial activity of seaweed S. duplicatum J. Agardh to reinforce that S. duplicatum J. Agardh seaweed has the potential as an alternative to natural preservatives in salted eggs. In this article we will discuss how to extract the ability of S. duplicatum J. Agardh to inhibit bacterial growth.

2. Experiments Procedure

Seaweed samples are stored in a cool box that has ice cubes to keep the seaweed fresh during the journey to the laboratory. Sargassum duplicatum seaweed preparation J. Agardh begins with washing, drying and grinding processes. The next stage is the extraction of the active ingredient. The extraction method used is a single extraction method that refers to Quinn [10]. The crushed sample was weighed 150 grams and

macerated with methanol solvent of 1100 mL for 48 hours. The result of maceration in the form of solution is then filtered with filter paper to obtain filtrate and residue. The obtained filtrate was evaporated using a Rotary Vacuum Evaporator at a temperature of less than 50°C so that the solvent separated with the extract. The results of this extract are then divided into two samples. The first sample was extracted without treatment (R_1). The second sample was an extract heated for 45 minutes over water bath to boil at 100°C (R_2). Both samples were tested in photochemistry, total phenol test, anti oxidant test and anti bacterial test. Phytochemical test to determine the presence or absence of bioactive components found in crude extracts Sargassum duplicatum J. Agardh. Phytochemical tests performed were modified from Harborne [11] which included steroid or triterpenoid, flavonoid, and saponin. The total content of phenol was determined using a modified Folin-Ciocalteau procedure from Pambayun [12] in which the total content of phenol test was performed to determine the amount of phenol present in the sample. The crude extract weighing about 5 mg was weighed and then dissolved with 2 mL of 96% ethanol. The solution was added 5 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent 50% (v/v). The mixture was allowed to stand for 5 min and added 1 mL of NaCO3 5% (w/v). The mixture is homogenized and incubated for 15 minutes. The resulting uptake was measured with a UV-Visible spectrophotometer at an 805 nm wavelength. Ergic acid was used as a standard with concentrations of 10, 15, 25, and 50 ppm.

The antioxidant activity test by DPPH method was first described by Blois. The antioxidant activity test performed in this study used a procedure, ie absorbance calculated from 1 ml sample mixed 1 ml DPPH and diluted with 2 ml methanol [13]. The Salmonella-Shigella inoculation antibody test is performed in aseptically sterile place by spraying 70%. 1 gram sample was dissolved with physiological solution (NaCl 0.85%) of 50 mL homogenized then put into a test tube already containing physiological solution 9 mL. 1 mL of the mixture was again fed into a test tube containing a 9 mL physiological solution, then taken 1 mL from each test tube and then fed into a Petri dish containing SSA medium. After 2x24 hours observed colony growth on SSA medium. The SSA medium is a selective medium, so that growing colonies can be expressed as Salmonella-Shigella colonies only. Confirmation of results, calculated by colony counter and gram stain on Salmonella-Shigella bacteria colony, where both of these bacteria are gram negative bacteria, this type of bacteria will give a red response. Salmonella shaped basil and Shighella in the form of kokobasil [14].

3. Result and Discussion

Phytochemical tests performed in this study include flavonoids, steroids or triterpenoids, and saponins. Photochemical test results can be seen in Table 1. In general phytochemical components contained in S. duplicatum extract J. Agardh are flavonoid and steroid / triterpenoid compounds.

Table 1. The results of phytochemical test of Seaweed extract *(Sargassum duplicatum* J. Agardh)

No	Phyto chemical Test	Without heating (R_1)	With heating (R_2)	Color (default)
1	Flavonoid	1. + 2. +	1. + 2. +	Amyl layer alcohol colored Red Yellow/ green
2	Saponin	1. + 2. +	1. + 2. +	Foam-shaped
3	Steroid/ Triterpen oid	1. + 2. +	1. + 2. +	Change Red becomes Blue / green

The total concentration of phenolate in the Seaweed Seed Extract S. duplicatum J. Agardh in the analysis with the standard curve, as in

Figure 1, is made as the equivalent comparator of phenolic compounds contained in seaweed extract (*Sargassum duplicatum J. Agardh*), thus the curve is useful. In helping to determine the total phenolic level. Research on standard solution of gallic acid yield regression equation y = 0.0078x + 0.0372 with coefficient of determination (R_2) equal to 0.9964. The coefficient of determination is a number whose value ranges from 0 to 1 indicating how closely the approximate value for the regression analysis represents the actual data. The regression analysis is most reliable if the value of R_2 is equal to or close to 1. The value of R_2 on the curve is 0.9964 so it can be used in the calculation of the content.

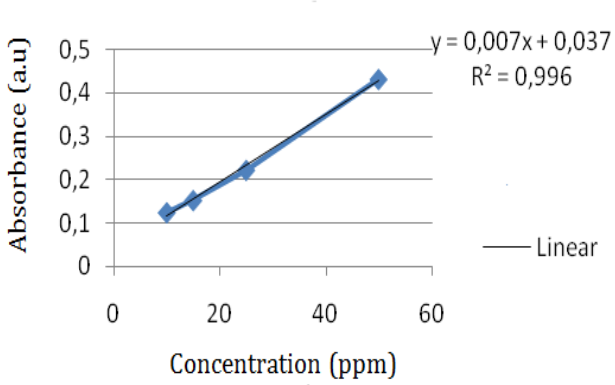


Figure 1. Standard Curve of Error Curve

The results of the study in Table 2 show that in the seaweed extract there is total phenolate. The total presence of this phenolate decreases with the warming done on the sample. The heating of the sample indirectly reduces the phenolate level because the bioactive compounds present in the seaweed extract are also damaged. These phenolic compounds contribute to antimicrobial activity [3]. This phenolic compound is also one type of antioxidant in food. Phenolic compounds proved to be an effective source of antioxidants, free radical retention, and chelating of metal ions. The antioxidant activity of phenolic compounds is related to phenol compounds [15].

Antioxidant activity on Seaweed Seed Extract was tested by absorbance measurements of several samples performed at concentrations or diluted 25 ppm, 50 ppm, 75 ppm and 100 ppm with repetition 2 times. This dilution aims to extend the range of concentrations with a constant susceptible so that the intersection points can be substituted as accurate linear equations, so that IC50 can derive from the equation [16]. The antioxidant activity test method using DPPH free radical was chosen because the method was simple, easy, fast, sensitive and required only a small sample, but the amount of diluent solvent required in this test was considerable. The solvent used is methanol. Methanol is chosen as a solvent because methanol can dissolve the DPPH crystals and also has properties that can dissolve non-polar components in them [13].

Table 2. Results of total phenol analysis in the extract (Sargassum duplicatum J. Agardh)

Methanol Extract	Absorbance	Concentration of Acid Errors (mg/L)	Total Phenolic Content (mg Equivalent Acid Error per 100 g of extract)
Without Heating	0,216	22, 92	9168
With Heating	0,174	17, 54	7016

A compound may be said to have antioxidant activity when the compound is able to donate its hydrogen atom to a DPPH radical characterized by a purple change to a pale yellow. The capture of these free radicals leads to diazo double bonds in the DPPH decreasing resulting in decreased absorbance. Antioxidant activity test was done quantitatively by using UV-Vis spectrophotometer. The test results are reported as IC50. IC50 is one of the commonly used parameters to interpret the results of DPPH testing. This IC50 value can be defined as the substrate concentration which can lead to a 50% reduction in DPPH activity. The

smaller the value of IC50 means higher antioxidant activity [13].

The amount of antioxidant activity is characterized by the value of IC50, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. The data of absorbance value and percent (%) inhibition of each sample extract can be seen in Table 3 and Table 4, while the more detailed percentage (%) inhibition calculation can be seen in the appendix. Calculation of percent (%) of inhibition begins by determining the maximum wavelength (λ_{max}) DPPH first. Based on Figure 2 obtained λ_{maks} of 515 nm.

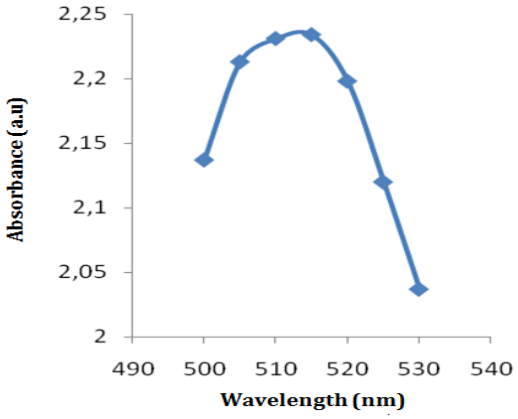


Figure 2 The Graph of antioxidant activity test was done quantitatively by using UV-Vis.

Table 3. Absorption and percent (%) Inhibition on sample R_1

No	Concentration (ppm)	R_1 repeat 1			R_1 repeat 2			mean % Inh
		A DPPH	A Sample	% Inh	A DPPH	A Sample	% Inh	
1.	25	0.675	0.471	30.22	0.676	0.473	30.03	30.12
2.	50	0.688	0.459	33.28	0.688	0.458	33.43	33.36
3.	75	0.647	0.410	36.63	0.649	0.411	36.67	36.65
4.	100	0.664	0.375	43.52	0.667	0.375	43.79	43.65

From Table 3 data can be substituted into the linear equation Y = aX + b. Linear equations can be seen in Figure 3.

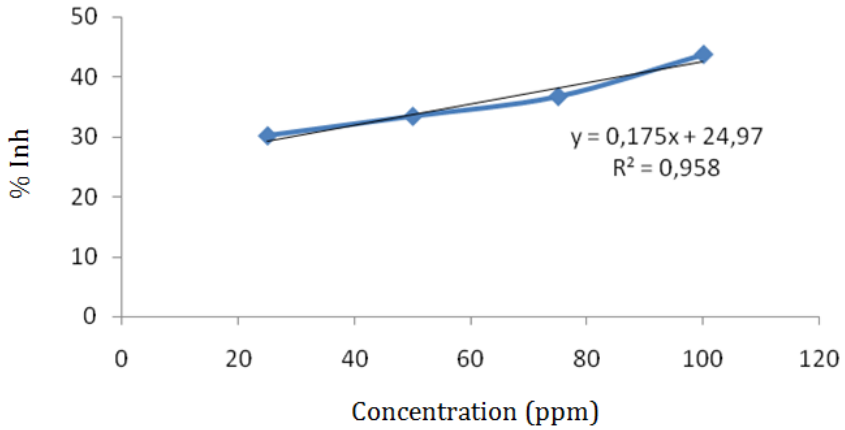


Figure 3. Grafik persen (%) Inhibisi ekstrak metanol pada sampel R_1

The linear equation is, y = 0.175x + 24.97

$$IC_{50} = \frac{50-24,97}{0,175} = 143.03\mu g/mL$$

Table 4. Absorption and percent (%) Inhibition on sample R_2

No	Concentration (ppm)	R_2 Repeat 1			R_2 repeat 2			Mean % Inh
		A DPPH	A Sample	% Inhibisi	A DPPH	A Sample	% Inh	
1.	25	0.681	0.440	35.39	0.685	0.440	35.77	35.58
2.	50	0.691	0.439	36.47	0.692	0.439	36.56	36.51
3.	75	0.650	0.410	36.92	0.653	0.412	36.91	36.91
4.	100	0.656	0.400	39.02	0.659	0.400	39.30	39.16

From Table 5 data can be substituted into the linear equation Y = aX + b. Linear equations can be seen in Figure 4.

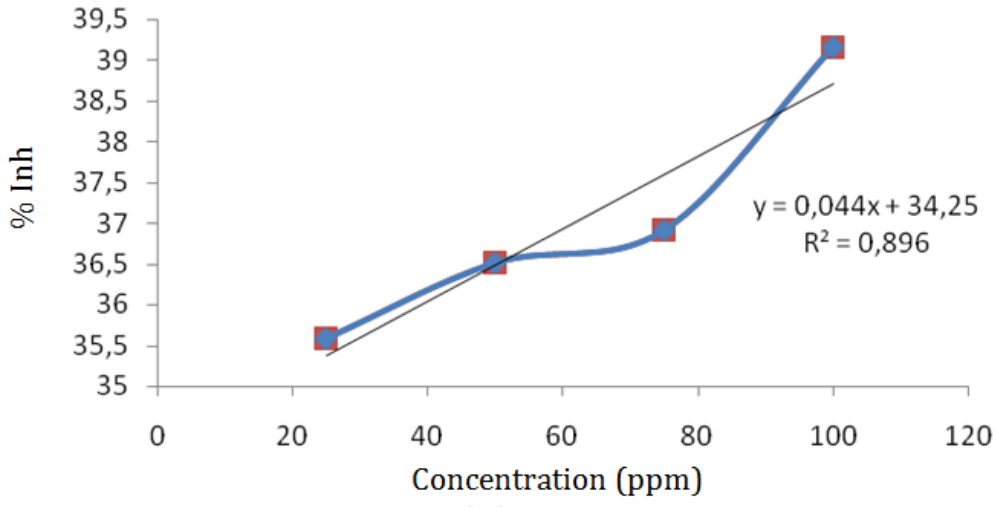


Figure 4. Graph of percent (%) Inhibition of methanol extract on sample R_2

The linear equation is, y = 0.044x + 34.25

$IC_{50} = \frac{50-34,25}{0,044} = 357.95 \mu g/mL$

Antioxidant activity is expressed in IC50. Based on the result of measurement of absorbance value and percent (%) inhibition can be obtained IC50 value from each methanol extract of seaweed S. Duplicatum J.Agardh. The higher concentration of extract used, the higher the percentage value of inhibition of free radical activity (percent inhibition). This correlates with the total phenol contained in the extract of S. duplicatum J.Agardh [3].

The compound is said to be a very powerful antioxidant if the IC50 value is less than 50 µg/ml, strong if the IC50 value is between 50-100 µg/ml, while the value of IC50 ranges from 100-150 µg/ml, and weak if IC50 value ranges between 150-200 µg/ml [17]. Based on the results of the study that the R_1 sample is classified as moderate antioxidants while the R_2 antioxidant sample is very weak.

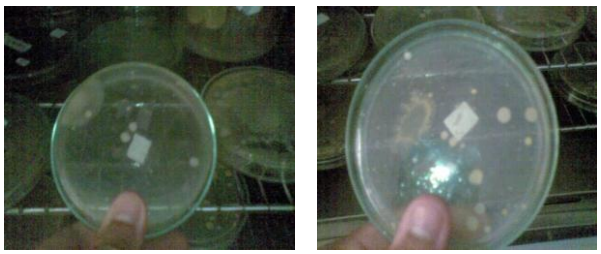
Different values of antioxidant activity occurred in both samples. Sample R_1 has a higher activity while the R_2 sample is lower. This is because the smaller the value of IC50 means higher antioxidant activity.

This decrease is also due to the heating done on sample R_2 so that there is damage to its bioactive compound. This correlates with the total phenol contained in the extract of S. duplicatum J. Agardh. This result is also in accordance with Molyneux [13]study which

states that if in a material has a high concentration of phenol compounds then the antioxidant activity in the material is also high.

Antibacterial test was conducted with the aim to find out how much inhibition power of S. seal extract S. duplicatum Agardh to bacteria. The bacteria used are Salmonella sp. The use of this bacterium due to contamination of pathogenic microorganisms that usually exist in eggs is the type of gram negative bacteria Salmonella sp [18]. This test was performed by diffusion method in order to Salmonella-Shigella Agar (SSA). The composition of this medium consists of peptone, lemco / beef extract lab, lactose, ox bile dried, sodium citrate, sodium thisulfate, ammonium iron (III) citrate, brilliant green, and neutral red agar, capable of inhibiting the growth of other bacteria, By using this selective medium only Salmonella-Shigella that grows and breeds.

The medium used is Salmonella-Shigella Agar (SSA). The use of this medium is capable of inhibiting the growth of salmonella-shigella that grows and breeds [19].



(a) **(b)**

Figure 5. Results of antibacterial activity test of methanol seaweed extract S. duplicatum J. Agardh against Salmonella sp. (a) repetition 1, (b) repetition 2 (Source: Laboratory Data of Nutrition and Food Sciences UNIMUS dated 13 April 2015).

Bacterial observations were performed by the gram staining principle which was then measured in a microscope. The results from Table 5 show that the SSA medium that has been added with the sample has a drag zone diameter of 1.12 mm and 1.15 mm. Controls in this test were used 10 µg chloramphenicol with a 10 mm inhibitory zone diameter. An antibacterial strength criterion is if the inhibitory zone diameter of 5 mm or less is categorized as weak, the inhibit zone 5-10 is categorized as medium, 10-20 zero inhibit zone is categorized as strong and the inhibition zone 20 mm or more is categorized very strong. This criterion is used in the study to classify the inhibitory control and sample test materials. Based on the measurement of inhibitory zone diameter extract on Salmonella sp. Bacteria, indicating that the antibacterial power possessed by seaweed extract is weak.

Table 5. The result of diameter zone inhibition of methanol extract of seaweed S. duplicatum J. Agardh against Salmonella sp. (A) repetition 1, (b) repetition 2.

Compound test	Inhibition zone diameter of bacterial growth (mm)	
	(a)	(b)
Chloramphenicol (control)	10	10
Sample	1.12	1.15

The small value of this inhibitory zone is probably due to the extract of flavonoids used in this study which is the result of the extraction process of the methanol solvent still contains other compounds that may not be antibacterial that can interfere with the antibacterial power of flavonoids. The ineffectiveness of methanol extract in inhibiting the growth of test bacteria is thought to be related to the semi-polar methanol properties, so that only a few bioactive components are dissolved in them. Sabir [20] in his research explains that flavonoid compounds have the ability to inhibit bacterial growth with several different mechanisms, including flavonoids causing damage to the permeability of bacterial walls, microsomes and lysosomes as a result of the interaction between flavonoids and bacterial DNA.

Steroid or steroidal compounds also have potential as an antibacterial compound. Steroid or steroidal compounds inhibit bacterial growth by the inhibition mechanism against protein synthesis because it

accumulates and causes the alteration of the components of the bacterial cell itself. The terpenoid compound easily soluble in lipid of this characteristic that make this compound more easily penetrate cell wall of Gram positive bacteria and Gram negative bacteria cell [21].

These antibacterial compounds will cause damage to the bacterial cell wall. This cell membrane destruction begins with the H + ions of the phenol compounds and their derivatives which will attack the polar group (phosphate group) so that the phospholipid molecule will break down into glycerol, carboxylic acid, and phosphoric acid. This results in phospholipids being unable to sustain the cell membrane form, consequently the membrane will leak and bacteria will inhibit even death [22,23]. The mechanism of the antibacterial compound in inhibiting microbial growth is divided into several ways, namely (1) altering the membrane permeability so that the destruction of the membrane will lead to inhibition of cell growth or the death of bacterial cells, (2) causing protein denaturation, (3) inhibiting the action of enzymes in cells Resulting in disruption of metabolism or the death of bacterial cells, and (4) damaging cell walls of microorganisms causing lysis [3].

The antibacterial activity test of seaweed extract S. duplicatum J. Agardh was tested on Salmonella sp bacteria [18]. This test is done by Agar Diffusion method because by this method the diffusion of extract on agar in the Petri dish will be better. Based on the Agar Diffusion method, antibacterial activity was determined by measuring the inhibitory diameter of the extract on Salmonella sp bacteria. Ie the clear area formed around the well. Inhibit zone values in S. j. Agardh S. duplicatum seaweed extract were 1.20 mm and 1.15 mm with positive control of 10 mm chloramphenicol.

Chloramphenicol is a commercial antimicrobial as a positive control that can inhibit the test bacteria (Staphylococcus aureus, including Salmonella sp.). Bioactive compounds are said to have a high activity on microbes if it has the lowest value of microbial inhibition, but has a large diameter of inhibition. The results of antibacterial activity test on Chloramphenicol showed a larger inhibitory zone diameter compared to S. duplicatum seaweed extract J. Agardh. This is because chloramphenicol is a pure antimicrobial compound while the seaweed extract S. duplicatum J. Agardh is still a crude extract containing organic material other than antibacterial [3].

The purpose of this antibacterial test is to find out how much antibacterial activity of crude extracts against Salmonella sp bacteria. The results of this test also gives an understanding if this extract has antibacterial properties against Salmonella sp. Bacteria, then this extract can be applied to the method of preservation of salted eggs. This suggests that if this crude extract is added to the salted egg it will increase its shelf life. Its working principle is with the antibacterial properties of the extract it will inhibit the growth of bacteria that cause decay in salted eggs that make eggs last longer.

The small value of this inhibitory zone is probably due to the extract used in this study which is the result of the extraction process from the methanol solvent still contains other compounds that can interfere with the antibacterial power of flavonoids. However, this lack of antibacterial power has not been able to fully imply that this extract can not be applied as a natural preservative in salted eggs.

The presence of phenolic group compounds in crude extracts such as flavonoids and steroids that are antibacterial, indicates that this seaweed extract has potential as an antibacterial. This is evidenced by the inhibition zone against Salmonella sp bacteria. On seaweed extract S. duplicatum J. Agardh. This implies that the rough extract of S. duplicatum seaweed S. Agardh has the potential to be developed as an alternative to natural preservatives in salted eggs.

4. Conclusion

The amount of antioxidant activity is characterized by the value of IC50. Sample R_1

yields IC50 value of 143,03 µg/mL, whereas in sample R_2 yield IC50 value equal to 357.95 µg/mL. Sample R_1 belongs to the moderate antioxidant category while the R_2 sample of antioxidants is very weak. Seaweed extract S. duplicatum J. Agardh is active as an antibacterial. This can be seen from the value of inhibitory power to the growth of Salmonella sp bacteria. The results showed that the diameter of the inhibitory zone of antimicrobial activity of methane seaweed extract of Sargassum duplicatum J. Agardh was 1.20 mm and 1.15 mm, Chloramphenicol was used as a control. The presence of phenolic group compounds in crude extracts such as flavonoids and steroids that are antibacterial, indicates that this seaweed extract has potential as an antibacterial. This is evidenced by the inhibition zone against Salmonella sp bacteria. In seaweed extract S. duplicatum J. Agardh. This implies that the rough extract of S. duplicatum seaweed S. Agardh has the potential to be developed as an alternative to natural preservatives in salted eggs.

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References

[1] N. Humphrey, How to solve the mind-body problem, *Journal of Consciousness Studies*, 7, pp.5-20, 2000.

[2] M.J. Pelczar M.J, Chan ECS, Krieg NR. .*Microbiology*. 5thEdn., Mcgraw-Hill College, 5261 Highland Road, PMB 339 Baton Rouge, La 70808, pp. 596-599, 1998.

[3] M. Renhoran,. *Aktivitas Antioksidan dan Antimikroba Ekstrak Sargassum Polycystum, Skripsi*, Bogor: Departemen Teknologi Hasil Perairan Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor, 2012.

[4] L.M. Aslan, *Budidaya Rumput Laut*, Yogyakarta: Kanisius, 1991.

[5] T. Setiana, Aisyah dan A. Asnani, *Kajian Sifat Fisikokimia Ekstrak Rumput Laut Coklat Sargassum duplicatum Menggunakan Berbagai Pelarut dan Metode Ekstraksi*, *Jurnal*, Purwokerto: Fakultas Pertanian Universitas Jenderal Soedirman, 2012.

[6] M. Sirait, *Penuntun Fitokimia dalam Farmasi,* Bandung: ITB, 2007.

[7] W.J. Tang, A.G. Gilman. Type-specific regulation of adenylyl cyclase by G protein βγ subunits. *Science 254*:1500–1503, 1991.

[8] M.S. Meskin, *Phytochemical in nutrition health,* London: CRC, 2002.

[9] S.R.Tamat, Aktivitas antioksidan dan toksisitas senyawa bioaktif dari ekstrak rumput laut hijau Ulva reticulate Forsskal. *Jurnal Ilmu Kefarmasian Indones,a,* Vol. 5, No. 1, 2007.

[10] L.K. Darusman L.K, Ekstraksi komponen bioaktif sebagai bahan obat dari karang-karangan, bunga karang dan ganggang laut di perairan Pulau Pari Kepulauan Seribu, *Buletin kimia*, Bogor: Institut Pertanian Bogor. 1995.

[11] J.B. Harborne, Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan, Alih bahasa Kosasih Padmawinata, Bandung: Penerbit ITB. 1987.

[12] R. Pambayun, dkk., Kandungan fenol dan sifat antibakteri dari berbagai jenis ekstrak produk gambir, (Uncaria gambir Roxb), *Majalah Farmasi Indonesia*, 18(3), 2007.

[13] P. Molyneux, The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Journal of Science Technology*, Volume 26, No. 2, 2004.

[14] M. Jawetz, dan Adelberg, *mikrobiologi kedokteran*, Alih Bahasa Nani Widorini, Jakarta: Salemba Medika. 2001.

[15] M.S. Meskin, dkk., *Phytochemical in nutrition health,* London: CRC, 2002.

[16] S. Sudirman, Aktivitas Antioksidan dan Komponen bioaktif kangkung air

(ipomoea aquatica forsk.), *Jurnal*, Bogor: Departemen Teknologi Hasil Perairan Fakultas Perikanan dan Ilmu Kelautan I PB. 2011.

[17] A. Apriandi, *Aktivitas Antioksidan dan Komponen Bioaktif Keong Ipong-Ipong (Fasciolaria salmo)*, *Skripsi*, Bogor: Departemen Teknologi Hasil Perairan Fakultas Perikanan dan Ilmu Kelautan, IPB. 2011.

[18] M. Anggraini, dan Sofi, *Kajian Sifat Fisik, Kimia dan Mikrobiologi Kuningtelur yang Ditambah Madu dengan Jenisdan Umur Telur yang Berbeda*, Skripsi, Bogor: Departemen ilmu produksi dan teknologi peternakan Fakultas peternakan,IPB, 2011.

[19] N. Afifah, *Uji Salmonella-Shigella pada Telur Ayam yang Disimpan pada Suhu dan Waktu yang Berbeda*, *Jurnal*, Riau: Program Studi Pendidikan Biologi, Universitas Pasir Pengaraian. 2013.

[20] A. Sabir, Aktivitas Antibakteri Flavonoid Propolis Trigona sp terhadap Bakteri Streptococcus mutans (in vitro), *Majalah Kedokteran Gigi* (Dent J), Vol. 38, No. 3. 2005.

[21] K. Rosyidah, Aktivitas Antibakteri Fraksi Saponin dari Kulit Batang Tumbuhan Kasturi Mangifera casturi, *Jurnal Alchemy*, Vol. 1, No. 2. 2010.

[22] P.F. Sari, dan S.M. Sari, Ekstraksi Zat Aktif Antimikroba dari Tanaman Yodium (Jatropha multifida Linn) sebagai Bahan Baku Alternatif Antibiotik Alami, *Jurnal*, Semarang: Jurusan Teknik Kimia Fakultas Teknik Universitas Diponegoro. 2012.

[23] L. Brunton, B.A. Chabner, B. Knollman, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th edition, McGraw-Hill Companies. 2011.