Food ScienTech Journal Vol. 1 (1) 2019 ISSN : 2685-4279 DOI:10.33512/fsj.v1i1.6244

PHENOLIC COMPOUND OF FRESH AND BOILED SEA GRAPES *(CAULERPA SP.)* FROM TUAL, MALUKU

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ABSTRACT

Caulerpa sp. commonly found in Tual, Southeast Maluku sea. *Caulerpa* sp. is generally consumed by coastal communities as raw food such as salads or processing food such as boiled *Caulerpa* sp. Nutrient information and total phenolic content of *Caulerpa* sp. during boiling has not been reported. The aim of this study is to determine bioactive components and total phenolic content from *Caulerpa* sp. as a result of the boiling process. The boiling process was carried out for 5 minutes at 90 ℃. The boiling process causes flavonoids loss in *Caulerpa* sp. Total phenolic content after boiling process decreased by 11.76 mg GAE/g extract. Total phenolic of fresh *Caulerpa* sp higher than the boiled *Caulerpa* sp.

Keywords: *Caulerpa* sp., phytochemicals, boiling, total phenolic content

INTRODUCTION

Maluku Province is geographically bordered by North Maluku province. In the east, it bordered by Southeast Sulawesi and Central Sulawesi, and in the south it bordered by East Timor and Australia. It is an archipelago province with an area of 712,479.65 km^2 consisting of 93.5% of the water area and 6.5% of the land area. The number of islands in Maluku reaches 1,340 islands. Tual City is a division of the Southeast Maluku district with an area of 19,088.29 km^2 with of 98.67% sea area and 1.33% land area. The main commodity in Tual city is seaweed (BKPMD, 2012).

Seaweed production in Maluku region reached 6.3 million tons in 2013 which included seaweed from the *Caulerpa* genus (2015 MPA). The people inform that *Caulerpa* sp. is used for consumption. *Caulerpa* sp. is generally consumed by coastal communities as raw food such as salads or processing food such as boiled *Caulerpa* sp. The communities considers that boiling process can kill the bacteria that might be found in *Caulerpa* sp., and it is done for five minutes.

Nurjanah *et al.* (2016) said that green seaweed *Caulerpa* sp could be as cosmetic material. Nurjanah *et al.* (2018) also stated that green seaweed *Caulerpa lentilifera* has high fiber content. *Caulerpa lentilifera* is potentially to produce low sodium salt and antioxidant (Nufus *et al.* 2017). Saputra *et al.* (2011) stated that seaweed *Caulerpa racemosa* is biogas material because it has high carbohydrate content. High and low content of carbohydrate affect biogas pressure, the higher the carbohydrate, the greater the biogas pressure was obtained. Adriani (2015) stated that *Caulerpa racemosa* contains flavonoid compounds that show antibacterial activity, because it produces endophytic fungi that can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria.

Phenolic compounds are a group of chemicals compounds in plants. The group of phenolic compounds has a role as antioxidant which can reduce the risk of heart disease, blood vessels, and cancer. Many phenolic compounds are found in fruits, green vegetables, and nuts. Phenolic compounds act to protect body cells from free radical damage by binding to the free radicals so that they can prevent inflammation in the body cells (Winarti, 2010).

Phenolic compounds include various compounds from plants that have aromatic rings with one or two hydroxyl groups. Some groups of polymeric materials in plants such as lignin, melanin, and tannins are phenolic compounds. Phenolic is a aromatic compounds, so that it show strong uptake in visible spectrum regions (Harborne, 1987). Cho *et al.* (2010) stated that the total phenolic content of fresh seaweed *Caulerpa lentillifera* with methanol extract is 6.7 mg GAE/g extract. But, Maulida (2007) implies that the amount of total phenolic content is 8.95 mg GAE/g extract.

Caulerpa sp regards as sources of phenolic compounds. The total content of phenolic compounds *Caulerpa sp.* during the boiling process needs to be considered, because this process can affect the physical and chemical materials conditions. Analysis of phenolic content of boiled *Caulerpa* sp. have not been reported, so that an analysis is needed to determine the total phenolic content of *Caulerpa* sp. after the boiling process. This study aims to determine the bioactive components qualitatively and the total content of phenolic from fresh and boiled seaweed *Caulerpa sp.* in a temperature of 90^{o}C for 5 minutes.

MATERIALS AND METHOD

Materials and Tools

The main ingredient was seaweed *Caulerpa sp*. The ingredients used for extraction were methanol and filter paper. The materials for proximate testing are hexane (Emsure) solvent, concentrated H_2SO_4 (Emsure), aquades, NaOH (Emsure), H_3BO_3 (Emsure), HCl (Emsure). Reagents for phytochemical analysis are sulfuric acid, dragendroff reagent, meyer, Wagner, chloroform (Emsure), anhydra acetate, concentrated sulfuric acid, magnesium, amyl alcohol, alcohol, ethanol, 5% $FeCl_3$, 2N HCl (Emsure). The materials for analysis were Folin-Ciocalteau (p.a) reagents, gallic acid (Yalong), ethanol (Emsure), aquades, and sodium carbonate (Yalong).

The sample preparation tools are knives, cutting boards, containers, and analytical scales. The proximate analysis tools are porcelain saucer, desiccator, oven, furnace, soxhlet tube, kjeldahl tube, flask, and erlenmeyer. The extraction tools are orbital shaker (WiseShake), rotary evaporator (Buchi Rot. R-205), and the phenolic analysis tools is Ultra Violet-Visible (UV-Vis) spectrophotometer (Hitachi U-2800).

Sample Preparation

Samples were taken from Tual waters, Maluku. Part of the sample taken is all. Samples were taken fresh, then packaged using plastic bags, plus sea water and then put into a jar, sent from Maluku to the Bogor Agricultural Institute, Bogor using air transportation modes. Fresh samples last for one week after being taken from the waters. Arriving in Bogor, the samples were left at room temperature, then prepared. Fresh seaweed is cleaned of dirt that is still attached, then weighed. Samples were divided into two namely, *Caulerpa* sp. fresh and boiled. The boiling temperature and time used refers to Putera's research (2015), which is the best boiling treatment at 90 °C and the boiling time 5 minutes. Boiling uses mineral water, with a sample and water ratio of 1: 4 (w / v). The water is heated first until it reaches a temperature of 90 °C, then *Caulerpa* sp. put in boiling water and counted up to 5 minutes. The next step is proximate analysis, phytochemical analysis and total phenolic compounds. The flow chart of the research procedure can be seen in Figure 1.

Bioactive Compound Extraction

The fresh *Caulerpa sp*. was mashed by mortar, then it is weighed. The boiled *Caulerpa sp.* was drained, mashed using a mortar, and weighed using scale. *Caulerpa sp.* was weighed as much as 100 g and put in the 500 mL of erlenmeyer. It is added 200 mL of methanol. The samples that have been submerged with methanol, then macerated by using an orbital shaker for 1x24 hours. The filtrate was then evaporated by using a rotary vacuum evaporator in 40 ^{o}C. The extraction was repeated three times.

Phytochemical Analysis (Harbone, 1987)

Phytochemical analysis was conducted to determine the presence or absence of bioactive components in fresh and boiled *Caulerpa sp*. which contains phenolic content. Phytochemical tests include alkaloid test, steroid / triterpenoid test, flavonoids, and phenolic hydroquinone.

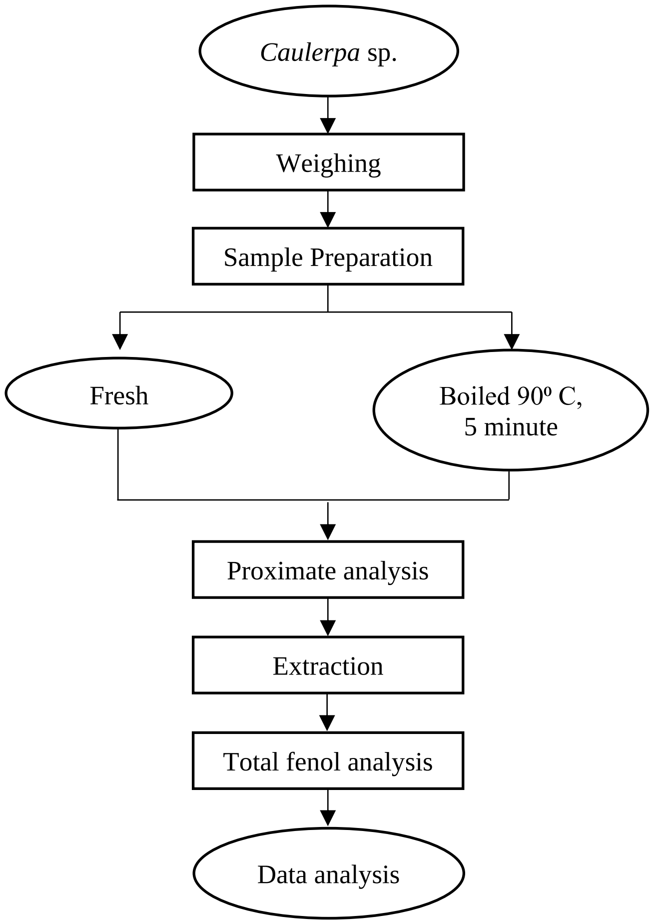

Figure 1. Flow chart

Total Phenolic Content (Modified Andarwulan, 2000)

Determination of the total phenolic content begins with making a blank solution and a gallic acid solution as a standard. A blank solution was made by piping 2 mL of 96% ethanol into 10 mL test tube. Gallic acid solution was made by preparing stock of solutions with a concentration of 100 ppm in 100 mL, 10 mg gallic acid dissolved in 50 mL 96% ethanol in a 60 mL extract bottle. The solution dilutes in a 10 mL test tube, with concentrations of 10, 30, 50, 70, and 100 ppm with a volume of 2 mL each.

The extract sample of *Caulerpa sp.* was weighed as much as 9.5 mg and dissolved with 2 mL 96% ethanol in a 10 mL test tube. Blank solution, standard solution or sample were added 5 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent 50% (v / v) and left it for 5 minutes. The solution was added 1 mL of sodium carbonate solution 5% (b / v) and homogenized and incubated in room temperature and in dark condition for 1 hour. Test the tubes before incubating them in aluminum foil to make the incubation effective in dark condition. The next stage after the incubation process was homogenized the solution then measured the total phenolic content using the Hitachi U-2008 UV-Vis spectrophotometer at a wavelength of 725 nm.

Data Analysis

Total phenol compounds were analyzed descriptively. Analysis of the data used for proximate values is a paired t test. This analysis aims to see whether there is an average difference from the *Caulerpa* sp. Sample. fresh and boiled against proximate values.

Hyphotesis:

H_0: There is no difference in the proximate value of *Caulerpa* sp. fresh and boiled

H_1: There is a difference in the proximate value of *Caulerpa* sp. fresh and boiled

Paired t test statistics are:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}}$$

Noted :

$\bar{d}$ = the average difference between the value of fresh and boiled proximate

s_d = standard deviation from the difference between fresh and boiled proximate values

n = the number of samples

RESULT AND DISCUSSION

Morphology and Proximate of *Caulerpa* sp.

Caulerpa sp. is a green seaweed from the *Caulerpaceae* family in which the habitat attaches to the substrate of sand or rocks. *Thallus* in *Caulerpa sp.* forms roots and *ramuli*. *Ramuli* forms small dots which regularly close over ± 3-5 cm of branching. The *Caulerpa sp.* condition used is fresh, but some parts of the ramuli are damaged. Tampubolon *et al.* (2013) stated that *Caulerpa lentillifera* has *thallus* with round propagate branches and the branches are like wine branch. The morphology of *Caulerpa sp.* can be seen in Figure 2.

Figure 2. *Caulerpa sp.*

Caulerpa sp. is used by coastal communities as consumption so that it is necessary to know the chemical and nutrient content of *Caulerpa sp.* Chemical composition of fresh and boiled *Caulerpa sp.* are presented in Table 1.

Table 1. Chemical composition of *Caulerpa sp.*

Chemical Composition	Fresh *Caulerpa* sp. Wet basic (%)	Boiled *Caulerpa* sp. Wet basic (%)
Water content	77,57±0,19	79,17±0,23
Ash content	1,18±0,15	1,02±0,01
Fat content	0,32 ± 0,01	0,37 ± 0,03
Protein content	3,84±0,04	3,63±0,06
Carbohydrate content *by different*	17,08±0,11	15,65±0,21

Water content in fresh *Caulerpa sp*. is 77.57 ± 0.19% and boiled *Caulerpa sp* 79.17 ± 0.23%. The t-test analysis result showed there are differences in the water content of fresh and boiled *Caulerpa sp.* (∝≤0.05). The difference of the water content between fresh and boiled *Caulerpa sp.* is due to the tissue in boiled *Caulerpa sp* can absorb water, so the water content of boiled *Caulerpa sp.* has increased. Aisyah *et al.* (2014) stated that the changes in the water content after boiling process is due to network matrices in vegetables which tend to absorb water, so that the water content is relatively higher than fresh vegetables. Kumar *et al.* (2011) stated that the water content of *Caulerpa racemosa* obtained from Indian waters is 91.53% and Ma'ruf *et al.* (2013) stated that the *Caulerpa racemosa* water content from Jepara waters is 92.37%.

The result studies show that the water content of fresh *Caulerpa sp.* is 77.57 ± 0.19%. The water content value is quite low compared to the previous studies. This difference is due to the lenghty of transportation process, and the condition of the sample is slightly open, so that the water in the sample is evaporated.

The ash content of fresh and boiled *Caulerpa sp.* are 1.18 ± 0.15% and 1.02 ± 0.01% respectively. The t-test analysis results show that there are not any differences in the ash content of fresh and boiled *Caulerpa sp.* (∝> 0.05). The boiling process affect the ash content in the material, because the minerals contained in *Caulerpa sp.* dissolve in boiling water. The boiling process also affect the ash content of the material, because of the influence of water vapor that comes out during the boiling process. Nurjanah *et al.* (2014a) states that the ash content of the genjer plant changes after steaming for 3 and 5 minutes. The minerals contained in the genjer plant emerge along with the discharge of water during steaming, so that the ash content of the genjer plants after steaming changes.

The fat content of fresh and boiled *Caulerpa sp.* are 0.32 ± 0.01% and 0.37 ± 0.02% respectively. The t-test analysis results show that there is no difference in the fat content of fresh and boiled *Caulerpa sp.*(∝> 0.05). The fat content of *Caulerpa sp.* is low due to the high water content in *Caulerpa sp.*, so that the fat content decreases. Kumar *et al.* (2011) stated that the fat content of fresh *Caulerpa racemosa* from Indian waters is 2.64%. McDermid and Stuercke (2003) stated that the fat content of fresh *Caulerpa lentillifera* obtained from the Hawaiian coast is relatively high in 7.2%.

The fat content value result from this sudy has differences with the previous research. The difference is caused by different environmental conditions, which are generally the harvest season and habitat of the biota. Wong and Cheung (2000) stated that the fat content in seaweed is low. The low fat content is caused by a high enough water content, so that proportionally the fat content will decrease.

The protein content of fresh and boiled *Caulerpa sp.* are 3.84 ± 0.04% and 3.63 ± 0.06%, respectively. The results of the t-test analysis show that there are differences in fresh and boiled *Caulerpa sp*. protein content (∝≤0.05). Boiling process affects the protein content which are due to the protein dissolves in the water that emerge from the material. Matanjun *et al.* (2009) stated that the fresh

Caulerpa lentillifera protein content are 10.41 ± 0.26%. This difference is caused by differences in habitat from the material used. Ratana and Chirapart (2006) stated that the different protein content in seaweed is caused by differences in species, season, and geographical conditions, as well as the amino acid content.

The difference in protein content after boiling is due to the presence of volatile nitrogen in seaweed proteins, which will evaporate because of heating process (Febriyanti 2011). The boiling period and temperature play a role in changing the nutritional content of food. The higher of the temperature and the less of boiling period will reduce the food nutritional content (Widyati 2001). Megayana *et al.* (2012) stated that the nutrient content that exists in the environment has an influence on the chemical composition of organisms living.

Carbohydrate content (by difference) of fresh and boiled *Caulerpa sp.* are 17.08 ± 0.11% and 15.65 ± 0.21%, respectively. The t-test analysis results show that there are differences in carbohydrate content of fresh and boiled *Caulerpa sp.* ($\propto \leq 0.05$). The changes in carbohydrates content of boiled *Caulerpa sp.* is caused by the nature of these carbohydrates which can dissolve in water. Carbohydrates content generally have links with fiber in a material. Kumar *et al.* (2011) stated that the carbohydrate content of fresh *Caulerpa racemosa* from Indian waters is 4.5%. Ma'ruf *et al.* (2013) stated that the high fiber content is caused by high polysaccharides in seaweed cells, which function as dietary fiber and functional fiber.

Phytochemicals from *Caulerpa* sp. Seaweed Extract

The yield of extracted fresh *Caulerpa sp*. is as much as 8.03% and yield of boiled *Caulerpa sp.* is 6.36%. Bioactive components in *Caulerpa sp*. are mostly soluble in polar solvents. The amount of yield affects the solubility properties of bioactive components. Kusumawati *et al.* (2008) stated that yield is the percentage of raw material that can be used. The larger yield indicates that the raw material has a greater chance to be used compared to raw materials which have a low yield value.

Plants contain many phenol compounds. These phenolic content tend to dissolve in polar solvents (Harborne 1987). The yield of extracts fresh *Caulerpa lentillifera* seaweed obtained from Teluk Betung, Lampung using methanol is 3.06% (Maulida 2007). This yields compare to the previous studies have far-reaching differences. This difference is expected because the method used, previous research using multilevel extraction methods and in this study using a single extraction.

Phytochemical analysis is carried out on fresh and boiled *Caulerpa sp.* to identify the chemical content as a first step in study the bioactive component. The compounds found in *Caulerpa sp.* tested qualitatively based on color changes or deposits formed in response to the given reagent. The presence of bioactive components *Caulerpa sp.* presented in Table 2.

Table 2. Chemical composition of *Caulerpa sp.*

Test	Seaweed *Caulerpa* sp.		Color change	
	Fresh	Boiled	Fresh	Boiled
Alkaloid				
Meyer	-	-	-	-
Wagner	-	-	-	-
Dragendroff	-	-	-	-
Steroid	+	+	Blue	Blue
Triterpenoid	-	-	-	-
Flavonoid	+	-	Yellow	-
Fenol hidroquinon	+	+	Bluish green	Green
Saponin	+	+	Foaming	Foaming
Tanin	-	-	-	-

Description:
(+) = Detected, (-) = Not detected

Phytochemical analysis showed positive results for the presence of steroid compounds in fresh and boiled *Caulerpa sp.* Steroid compounds are soluble in lipids and have potential as antibacterial compounds. The presence of steroid compounds in *Caulerpa sp.* according to Ahdyanti (2009) is fresh *Caulerpa racemosa* contains steroid and triterpenoid compounds.

Rosyidah *et al.* (2010) stated that steroid / triterpenoid compounds have activity as antibacterial compounds. Steroid / triterpenoid compounds can inhibit bacterial growth with an inhibitory mechanism for protein synthesis because it accumulates and causes changes in the constituent components of bacterial cells. Steroid / triterpenoid compounds are easily soluble in lipids, these factors can cause steroid / triterpenoid compounds easily penetrate bacterial cell walls.

Fresh *Caulerpa sp.* shows positive results on flavonoid compounds and boiled *Caulerpa sp.* has no flavonoid compounds detected. The boiling process can eliminate flavonoids. It is because flavonoid compounds dissolve in boiling water or because of an oxidation process occurs. The results of Lusivera (2002) study suggest that the boiling process resulted in negative flavonoid compounds in the binahong leaves. The flavonoid content in the binahong leaves decreased by 78%.

The presence of sugar bound to flavonoids causes flavonoids to dissolve easily in water (Markham 1988). Flavonoids are compounds that show biochemical activity, namely antioxidants, antiviral, antibacterial, and anticancer. Flavonoids are a group of phenolic compounds with chemical structures C_6-C_3-C_6 (Vermerris & Nicholson 2006). Sabir (2005) stated that flavonoid compounds able to inhibit bacterial growth. The inhibiting mechanism of these bacteria is the hydroxyl group found in the structure of flavonoid compounds causes changes in organic components and nutrient transport. Eventually, this lead to the emergence of toxic effects on bacteria.

Fresh and boiled *Caulerpa sp.* show positive results for phenol hydroquinone compounds. Phenolic compounds are found in plants. They are soluble in polar solvents. Phenolic compounds function as antioxidants. Nurjanah *et al.* (2014b) stated that water spinach plants contain phenolic compounds which are soluble in polar solvents. Phenolic compounds detected in water spinach have antioxidant activity.

Phenolic compounds tend to be soluble in water because these compounds bind to sugar as a glycoside and are usually found in cell vacuoles (Harbone 1987). Chen and Blumberg (2007) stated that phenolic compounds can reduce the risk of some chronic diseases because it characterize inflammatory, antioxidant, carcinogenic detoxification, and anti-cholesterol.

Fresh and boiled *Caulerpa sp.* show positive results on saponin compounds. Saponins generally dissolve in water and are slightly soluble in polar solvents. Complete hydrolyzed saponins produce sugar and one non-sugar fraction. Nurjanah *et al.* (2014a) stated that fresh genjer plants contain saponins and reducing sugars. The boiling process does not change the saponin and sugar contents. Koche *et al.* (2010) stated that the primary part of plant metabolism includes reducing sugars, amino acids, proteins, and chlorophyl.

The bioactive components in the material have various benefits. Nurjanah *et al.* (2009) showed phytochemical component analysis of sea leech flour extract is alkaloid compounds, saponins, steroids and phenols which were play a role in reducing total cholesterol and low density lipoprotein (LDL). Ruiz *et al.* (2005) stated that saponins function as antimicrobial compounds, anti-inflammatory and have low toxicity.

Groups of phenolic compounds include phenolic acids, simple phenols, flavonoids, anthocyanins, lignin, and tannins (Vermerris and Nicholson 2006). The results of phytochemical analysis in fresh and boiled *Caulerpa sp.* indicate the presence of phenolic compounds. Fresh and boiled *Caulerpa sp.*are positive against flavonoids and phenol hydroquinone compounds.

Total Phenolic Compound

Phenolic compounds are compounds that are widely found in plant species. They are consist of aromatic groups that absorb strongly in the spectrum of UV light. Reagent used to determine the presence of phenolic compounds in a material is Folin Ciocalteu (Harborne 1987). The total phenol analysis is conducted to determine the amount of phenol found in fresh and boiled *Caulerpa sp.* The total phenolic content of fresh *Caulerpa sp.* is 28.56 ± 1.97 mg GAE / g extract and in boiled *Caulerpa sp.* is 16.8 ± 1.53 mg GAE/g extract. The boiling process causes the total phenolic content of *Caulerpa sp.* decreases. It is because the total phenolic content of *Caulerpa sp.* is oxidized or dissolved in cooking water.

Aisyah *et al.* (2014) stated that the total phenolic content in vegetables after boiling process decreased by 13-16%. The boiling process will result in a total loss of phenolic compounds which can occur in two ways: dissolving in water during boiling and oxidation. Cho *et al.* (2010) stated that the total phenolic content of fresh *Caulerpa lentillifera* with methanol extract is as much as 6.7 mg GAE / g extract and Matanjun *et al.* (2008) stated that the total phenolic content in *Caulerpa lentillifera* is 42.85 mg GAE/g extract.

There are high differences between the total phenolic content result from this study compared to the existing literature. This difference is due to environmental factors and differences in *Caulerpa sp.* habitat. Reyes and Luis (2003) stated that sunlight is one of stressors that can increase biosynthesis of phenolic compounds in plant tissues.

The total phenolic compounds are influenced by sunlight, habitat, harvesting time, herbivore presence, and plant health conditions (Fithriani 2009). Phenolic compounds can be oxidized in alkaline solutions or because of the enzyme polyphenol oxidase activity that forms orthosemiquinone radicals that are reactive and react with amino compounds (Pratt 1992).

Prior *et al.* (2005) stated that the Folin-Ciocalteu method provides an estimated total phenolic compound value of the total phenolic content analyzed. Phenolic compounds are bioactive components that function as antioxidants. Jimenez-Escrig *et al.* (2001) stated that phenolic compounds are medically known as antitumor, hypo-allergenic and anti-inflammatory compounds.

Phenolic curcumin compounds from turmeric and phenolic catechins from tea are protective against gastric and intestinal cancers (Arnelia 2006). The role of phenolic compounds in the fisheries is to improve the characteristics of catfish surimi gel. The phenolic compound is obtained from dried tea leaf extract (Wijayanti 2012). Andayani *et al.* (2008) stated that research in plants reported that many plants contained large amounts of antioxidants. The effects of antioxidant was caused by the presence of phenolic compounds such as flavonoids and phenolic acids. Compounds that have antioxidant activity are phenolic compounds which have hydroxy groups substituted in ortho position and para -OH and -OR groups.

CONCLUSION

The water content after boiling process has increased by 1.6%, while the protein content and the carbohydrate content after boiling process have decreased by 0.21% and 1.43% respectively. Bioactive components found in boiled *Caulerpa sp.* are steroids, phenol hydroquinone, and saponins. The boiling process with a temperature of 90^{o}Cfor 5 minutes causes the loss of flavonoids which is one of the groups of phenolic compounds. Total phenolic compounds during the boiling process decrease as much as 11.76 mg GAE / g extract.

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