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Secondary Metabolite Compounds Potential (Tannins, Flavonoids, Alkaloids, Saponins) from Soft Coral of *Sarcophyton* sp. Living in the Waters of Lara Island, South Konawe Regency, Southeast Sulawesi

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ABSTRACT

This study aimed to determine the content of secondary metabolites, including tannins, flavonoids, alkaloids, and saponins, in the soft coral of *Sarcophyton* sp. in the three stations of different water depth of soft coral habitat at Lara Island waters, Southeast Sulawesi. The soft coral samples were taken at the depths of 3 meters (station I), 6 meters (Station II), and 9 meters (station III) using scuba equipment. The soft coral samples were placed in sample bags and a cool box, and brough to the Laboratory of Forensic Laboratory, Halu Oleo University. The samples then prepared and analyzed using spectrophotometric methods. The results showed that the concentrations of secondary metabolites in *Sarcophyton* sp. decreased as an increasing of water depth. The tannin contents were 0.538%, 0.367%, and 0.278% in waters depth of 3 m, 6m and 9m, respectively. Similar trend was found for flavonoid contents, i.e. 0.467% in 3m depth, 0.430% in 6m depth, and 0.343% in 9m depth. Moreover, alkaloid contents were 0.826%, 0.604%, and 0.522% respective in 3m, 6m and 9m depths. Meanwhile, saponin contents were found as 0.920% in 3m depth, 0.743% in 6m depth, and 0.520% in 9m depth. These results indicate that secondary metabolites compounds production of soft coral of *Sarcophyton* sp living at different depths were associated with environmental condition, particularly salinity levels, suggesting that environmental conditions play an important role in the biosynthesis of bioactive compounds in soft corals.

Keywords: *Environmental condition; Secondary metabolites; Soft coral; Sarcophyton* sp; Lara Island; Southeast Sulawesi

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INTRODUCTION

Indonesia is located at the center of the world's coral diversity, also known as the Coral Triangle (*Veron, 2000*). The coral coverage in Indonesia is estimated to be 51,000 km², or about 12.15% of the total coral coverage in the world, with 60% of the world's coral species found in this country. This coral reef area represents 18% of the total coral reef area in the world. More than 2,500 species of mollusks, 2,000 species of crustaceans, six species of sea turtles, 30 marine mammals, and more than 2,500 species of marine fish inhabit coral reef ecosystems (*Dahuri, 2003*).

Coral reefs are spread throughout Indonesia, including Southeast Sulawesi. Southeast Sulawesi boasts abundant aquatic resources and diverse ecosystems, including mangroves, seagrass beds, and coral reefs. Data reported by the TNC-WWF-Reef Cheek network indicates that 396 species of stony coral (consisting of 13 families) make up coral reefs.

Coral reefs are unique ecosystems found in tropical waters with high primary productivity and diversity, thus coral reefs play a very significant role in aquatic ecosystems. Ecologically, this ecosystem plays a crucial role in a body of water, namely as a habitat for foraging (feeding ground), spawning (spawning ground), and growing

(nursery ground) for various marine biota. Coral reefs are ecosystems on the tropical seabed built primarily by marine biota that produce lime ($CaCO_3$) ($CaCO_3$), also known as hermatypic corals.

In general, coral ecosystems can be divided into two main groups: hard corals and soft corals. Soft corals are the second largest group after hard corals and do not contribute to reef formation, but they have the same ecological role (*Manuputty, 1996*). This role can function well if environmental factors that support coral growth are not disturbed. Coral growth is greatly influenced by physical and chemical factors in waters such as nutrients, salinity, light, temperature, pollutants, and so on (*Suharsono 2010*).

Soft corals are among the soft animals that build coral reefs and can produce chemical compounds in the form of secondary metabolites (*Rumengan, 2013*). Soft corals are a rich source of bioactive compounds such as terpenoids, steroids, steroid glycosides, alkaloids, flavonoids, phenols, saponins, and peptides. Many secondary metabolites produced by soft corals have biological activities such as antifungal, cytotoxic, antineoplastic, antimicrobial, HIV inhibitor, and anti-inflammatory (*Radhika, 2006*).

Environmental factors significantly influence the production of secondary metabolites. Environmental stress and predator attacks will encourage aquatic organisms (including soft corals) to produce secondary metabolites to defend themselves against those stresses. Relatively dirty aquatic environments will have relatively high bioactivity, while relatively clean waters will have relatively low bioactivity and may even be inactive (*Rachmaniar, 1995*).

Sarcophyton sp. is a type of soft coral that produces secondary metabolites. *Sarcophyton* sp. is a type of soft coral with the potential to be used as a source of antioxidants. *Hardiningtyas* (*2009)* reported that the soft coral *Sarcophyton* sp. has antibacterial properties. Research conducted by *Sawant et al*. (*2006*) demonstrated antibacterial, antifungal, antitumor, neurotoxic, and anti-inflammatory activities. Research on the content of secondary metabolites (tannins, flavonoids, alkaloids, saponins) in the soft coral *Sarcophyton* sp. has never been conducted. Therefore, this study aimed to determine the content of secondary metabolites, including tannins, flavonoids, alkaloids, and saponins, in the soft coral of *Sarcophyton* sp. collected from different depth locations in the Lara Island waters, Southeast Sulawesi**.**

RESEARCH METHODS

Experimental design

Literature

This study determined the contents of secondary metabolites (tannins, flavonoids, alkaloids, and saponins) that produced by *Sarcophyton* sp. (soft corals) living at different depths in the waters of Lara Island, South Konawe Regency, Southeast Sulawesi.

Field Survey

The field survey was conducted in the coral reef ecosystem area of Lara Island, South Konawe Regency, Southeast Sulawesi by observing the depth of waters and the presence of *Sarcophyton* sp. Three stations were identified as the main sampling sites, i.e. station I is located at coordinates of 04°10166S and 122°66787 E, station II is located at coordinates of 4°6'8.12989S and 122°40'3.54612E, and Station III is located at coordinates of 4°6'8.53615S and 122°40'3.66261E.

Environmental parameters measurement

The environmental parameters, such as temperature, salinity, pH, and dissolved oxygen (DO) were measured in each station by standard method. Three replications were taken in each research station for each environmental parameter.

Soft coral sampling

Soft coral sampling was conducted by using the exploration method. The soft coral samples were taken based on their occurrence at the depths of 3 meters, 6 meters, and 9 meters using scuba diver. The samples of soft coral were placed in sample bags then putted them in a cool box, and then transported to the Biology Laboratory of the Forensic Unit, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, for analysis.

Sample Extraction

A 50 g sample of soft coral was crushed using a blender until smooth. Once smooth, the soft coral sample was placed in a beaker and macerated using a 95% methanol solution for 3 days (72 hours). After that the samples were extracted and then oven-dried for 1 day (24 hours). The extract then filtered and analyzed for its secondary metabolite compounds, consisting of tannins, alkaloids, saponins, and flavonoids as following procedures:

Tannin Content Analysis

The tannin content of soft coral is determined using a spectrophotometric method developed by Rangana, 1979. To do this, a standard curve is created by dissolving 0.5 mg of tannic acid in 50 mL of distilled water. Then, a standard solution of tannic acid with concentrations ranging from 0.02 to 0.1 mg/mL is prepared, followed by the addition of 0.5 Folin Denis reagent and 1 mL of saturated sodium carbonate solution, and made up to 10 mL with the addition of distilled water. The mixture is then thoroughly mixed and its absorbance is measured at a wavelength of 725 nm using a blank solution showing an absorbance of 0 (zero). The tannin content of the sample is determined using the standard curve. The tannin content is analyzed according to the method of Rangana (1976).

$$\text{Tannin as tannic acid (\%)} = \frac{\text{mg tannic acid} \times \text{dilluent} \times 100}{\text{mL of sample measured} \times \text{weight of sample} \times 1000}$$

Analysis of Flavonoid Content

A 200-ppm quercetin solution was diluted in 80% ethanol to obtain concentrations of 2: 4: 6: 8: ug/mL. The standard dilution solution (0.05) was mixed separately with 1.5 mL of 96% ethanol, 0.1 mL of A1C13, 1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at a wavelength of 428 nm using a UV-Vis spectrophotometer. A certain amount of 10% aluminum chloride was replaced with a certain amount of distilled water in the blank. Furthermore, 0.2 grams of sample was diluted with 80% ethanol. The sample was then added with 1.5 mL of 96% ethanol, 0.1 mL of 10% A1C13, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was then allowed to stand for 30 minutes at room temperature. The absorbance of the solution was measured at a wavelength of 428 nm. Flavonoid levels were determined based on a quercetin standard curve (Musdalifa, 2018).

Alkaloid Content Analysis

Alkaloid content analysis was performed by weighing 0.2 grams of soft coral sample, then placing it in a test tube with 10% acetic acid and letting it sit for 4 hours. The sample was then filtered using filter paper and the filtrate was concentrated using a hot plate until the volume was reduced to ¼. After that, it is removed and left to cool, then concentrated ammonium hydroxide solution is added to the sample extract until the sediment is complete, then left for several minutes and then filtered and the residue is dried. The alkaloid content is calculated by drying the residue from the soft coral extraction, then weighing the residue (weight of filter paper + weight of residue) - initial weight of paper.

Saponin Content Analysis

Saponin content analysis was done by reflux 0.2 grams of extract with 50 mL of petroleum ether at a temperature of 60-80°C for 30 minutes. After cooling, discard the petroleum ether solution and dissolve the residue in 50 mL of ethyl acetate, and the transferred the solution to a funnel and separate the ethyl acetate solution. The remaining residue was dissolved with butanol three times, each time with 50 mL All butanolic solutions were mixed and evaporated with a rotary evaporator. The evaporation residue was dissolved with 10 mL of

methanol, then this solution was dripped into 50 mL of diethyl ether while stirring. The precipitate formed in the mixture is poured onto filter paper of known weight. The precipitate on the filter paper is then rinsed with 10 mL of diethyl ether. The filter paper is dried and weighed until the weight remains constant. The difference in weight of the filter paper before and after filtration is determined as the weight of saponin.

$$\frac{X2-X1)}{A} \times 100\ \% = \%$$

Where: X1 is the weight of filter paper (g), X2 is the weight of filter paper + saponin sediment (g), and A is the weight of sample (g)

Environmental Parameter Measurement

Environmental parameters of coastal water in different depth as the habitat of soft coral *Sarcophyton* sp were measured including temperature, salinity, pH and Dissolved oxygen (DO). The depth of the soft coral habitat was measured by a raffia rope tied to a stone was submerged to the seabed and then measured using a meter. Temperature measurements were taken using a thermometer in the research area. Sea water temperature measurements were taken by dipping the tip of the thermometer into the sea water at depths of 3 meters, 6 meters, and 9 meters for approximately 15 minutes, then recording the results on the scale.

To determine the salinity level of seawater, a drop of seawater is taken at depths of 3 meters, 6 meters, and 9 meters at the research location. The seawater is then placed in a hand refractometer and the scale reading is observed. On the other hand, to determine the pH of seawater, measure the pH value using a pH meter by dipping the electrode into seawater at depths of 3 meters, 6 meters, and 9 meters, then observe the scale values displayed on the pH meter. However, dissolved oxygen (DO) measurements were done by using a DO meter by immersing the device in seawater samples at depths of 3 meters, 6 meters, and 9 meters, then recording the readings obtained.

Data Analysis

The obtained data of tannin, alkaloid, flavonoid, and saponin contents, were statistical analysis using the t-test using Excell. Data in the form diagrams were displayed using Kaleidagraph version 4.0 software.

RESULTS AND DISCUSSION

Environmental condition

The environmental condition of waters as the habitat of soft coral (*Sarcophyton* sp) in Lara Island, North Moramo District, South Konawe Regency, Southeast Sulawesi showed variation (Table 1) including temperature, salinity, pH and dissolved oxygen (DO) as an increasing of water deep. The temperature values were measured as 30°C, 27°C, and 26°Cat Stations I, II, and III, respectively. The salinity levels showed increased, i.e. 29‰ at Station I, 30‰ at Station II, and 32‰ at Station III, while pH seemed to show a decreasing trend from 8.21 at Station I, 7.89 at Station II, and 7.85 at Station III. However, dissolved oxygen (DO) concentrations were found at increasing trends, which were at level of 7.12 mL/L at Station I, 7.56 mL/L at Station II, and 7.89 mL/L at Station III, respectively. Therefore, water depths affect environmental condition of soft coral habitat in the present study site. Lower water depth (3 m) indicates high temperature and pH, while deeper water station (9 m depth) showed high salinity and DO.

Environmental conditions, particularly salinity, play a crucial role in regulating the production of secondary metabolites in soft corals. Salinity stress can trigger a biochemical defense mechanism that enhances the synthesis of protective compounds. According to Mayer (2010) and Radhika (2006), soft corals produce bioactive compounds such as terpenoids, flavonoids, and alkaloids as chemical defenses against environmental pressure and predation. Low-salinity environments impose osmotic stress, which can increase the accumulation of bioactive compounds as part of coral adaptation mechanisms.

Table 1. Average Values of Environmental Parameters at the Research Stations of Sof coral habitat in Lara Island

Stations	Environmental Parameters			
	Temperature	Salinity	pH	DO
I (3m)	30 (^{0}C)	29 ($^o/_{oo}$)	8,21	7,12 (ml/L)
II (6m)	27 (^{0}C)	30 ($^o/_{oo}$)	7,89	7,56 (ml/L)
III (9m)	26 (^{0}C)	32 ($^o/_{oo}$)	7,85	7,89 (ml/L)

Secondary Metabolic Compound

Tannin Compound Content of *Sarcophyton* sp. Soft Coral

The tannin content in *Sarcophyton* sp. soft corals (Figure 1) varied among stations ($P<0.05$), which was higher at Station I (0.53%) than Station II (0.36%) and Station III (0.27%). This condition indicates that environmental factors play a major role in the formation of tannin compounds. Field observations showed that Station I had previously experienced environmentally unfriendly fishing activities, causing long-term pollution. This pollution had an impact on environmental conditions, which became unstable for soft coral growth. Extreme environments, such as low salinity, can cause the formation of free radicals in the water. Romansyah (2011) explains that free radicals can be formed as a result of environmental pollution and can damage coral cells and tissues, thereby disrupting their physiological processes. To overcome this impact, organisms need antioxidant compounds as protection.

Tannins are known as antioxidant compounds that play an important role in neutralizing free radicals. According to Sunarni (2005), antioxidants can inhibit the action of free radicals by donating one or more electrons, thereby stabilizing free radicals and rendering them non-reactive. Aini (2007) adds that antioxidants are capable of donating hydrogen ions to free radicals, thereby forming more stable compounds. Thus, the high tannin content at Station I is thought to be a form of biological adaptation of the soft coral *Sarcophyton* sp. to abnormal environmental conditions, particularly due to low salinity stress and exposure to pollutants.

Low salinity conditions can disrupt the osmotic balance of coral cells and cause physiological disturbances. In response to this pressure, corals will produce more secondary metabolites, including tannins, to defend themselves. Muniarsih (2005) states that high levels of secondary metabolites in marine organisms are greatly influenced by environmental conditions such as salt content, light intensity, currents, and the level of competition between organisms. Dedi et al. (2017) reported that changes in salinity can cause damage to important cells in the coral body, thereby disrupting its growth.

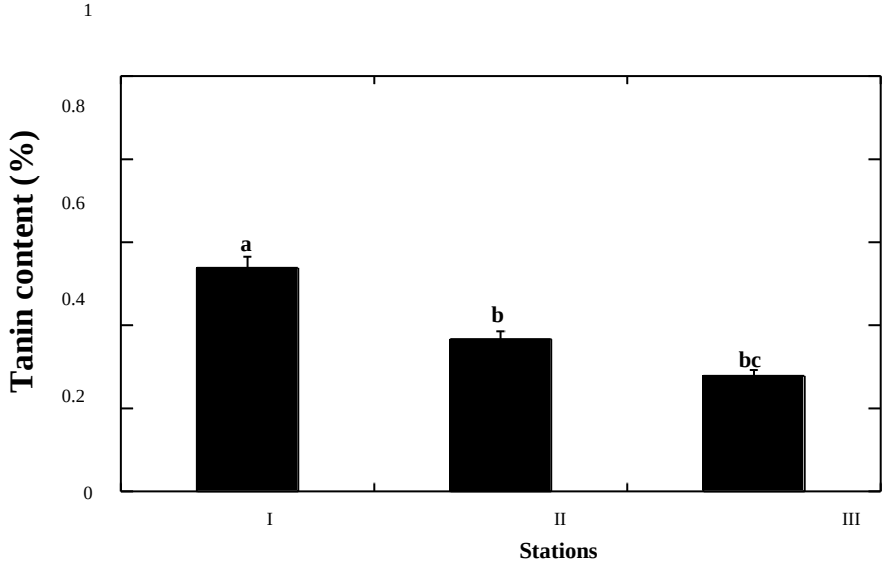

Figure 1. Histogram of tannin content at each research station of *Sarcophyton* sp. Soft coral

Flavonoid Compound Content of *Sarcophyton* sp. Soft Coral

The flavonoid compound content in *Sarcophyton* sp. soft coral varied among research stations ($P<0.05$), which was 0.46% at station I, 0.43% at station II, and 0.34% at station III. The highest flavonoid content was found at station I, while the lowest flavonoid content was found at station III. The salinity level at station I is relatively lower than at stations II and III, which might be induced the soft corals to produce secondary metabolites as a means of surviving low salinity stress conditions. Sholehah (2016) states that different aquatic environmental factors (low salinity stress) in each region will affect the formation of secondary metabolites. However, the environmental factors greatly influence the formation of secondary metabolites, one of which is flavonoids. It seemed that low salinity levels may cause free radicals. Flavonoids are compounds that have more complex phenol groups with higher degrees of hydroxylation. The presence of hydroxyl groups in phenol and flavonoid compounds causes antioxidant activity. This may be because the oxygen atom in the hydroxyl group has enough free electron pairs to inhibit the reactivity of the reactive atoms that make up free radical compounds (Eleanore, 2013). Thus, the high flavonoid content at station I will help inhibit the entry of free radicals into soft corals.

Flavonoids are compounds that function as antimicrobials and antioxidants. Flavonoids are an important group because they have a broad spectrum of antimicrobial activity and can reduce immunity in target organisms (Naidu, 2002). The antibacterial properties of flavonoid compounds cause protein denaturation within cells. Scheuer (1994) added that the presence of flavonoid compounds in bacterial cells causes the OH group in flavonoids to bind with internal proteins in the bacterial cell membrane. This causes uncontrolled Na+ ion influx into bacterial cells. This causes the bacterial cell membrane to rupture, resulting in the death or lysis of the bacteria.

The high flavonoid content at Station I is thought to be a form of adaptation of soft corals to low salinity stress. Environmental conditions that are not conducive to soft coral life will trigger soft corals to produce secondary metabolites as a form of self-defense against extreme environmental conditions. This is in accordance with the statement by Nurfitriani, et al. (2017), that marine organisms produce secondary metabolites as a chemical defense mechanism to survive in their environment.

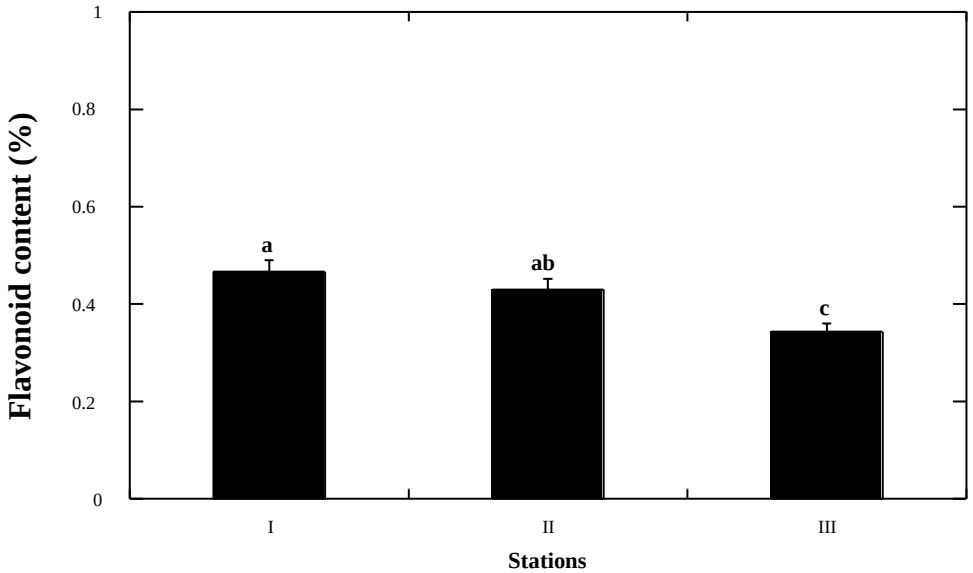

Figure 2. Histogram of flavonoid content at each research station of *Sarcophyton* sp. Soft coral

Alkaloid Compound Content of *Sarcophyton* Sp. Soft Coral

The alkaloid content of *Sarcophyton* sp. soft coral was estimated as 0.82%, 0.60%, and 0.52%. respective at stations I, II and III. Statistical analysis by student t-test showed that the alkaloid content at station I was significantly higher ($P<0.05$) than that at station III. It seemed that high alkaloid at station I was due to the salinity level at this station being below normal limits. Low salinity levels trigger soft corals to produce more alkaloid compounds at this station. The production of these alkaloid compounds is thought to be a form of self- defense by soft corals against low water salinity conditions. This is reinforced by Romansyah (2011), who states that soft corals produce alkaloid compounds that function as self-defense, infection prevention, and competition for space. However, water salinity influences the production of secondary metabolites. The lower the salinity of the water, the higher the level of secondary metabolites in soft corals. Hardiyanti (2011) said that environmental

factors greatly influence the production of secondary metabolites; organisms living in environments with very high levels of natural competition will have higher toxin content than organisms living in optimal environments.

Salinity conditions at station I are below normal thresholds due to effect of intensive human activity in this site. Low salinity levels make it difficult for soft corals to grow. Environmental conditions that are not conducive to soft coral growth will trigger soft corals to produce secondary metabolites, including alkaloids, as a form of adaptation to extreme environmental conditions (salinity stress). According to Supriharyono (2000), the resistance of coral animals to salinity levels has a threshold and is not the same for each species. Meanwhile, the best coral reef growth can be found in the salinity range of 34o/oo to 36o/oo (Ardiansyah, et al., 2013). Therefore, environmental conditions play a very important role in producing secondary metabolite compounds. marine environmental conditions greatly influence the active compounds produced in each species of soft coral, which support the soft corals to survive in different environmental conditions, especially low salinity stress, which can cause free radicals to enter the soft coral's body (Yulianti (2009, Wijayanti, et al. (2017).

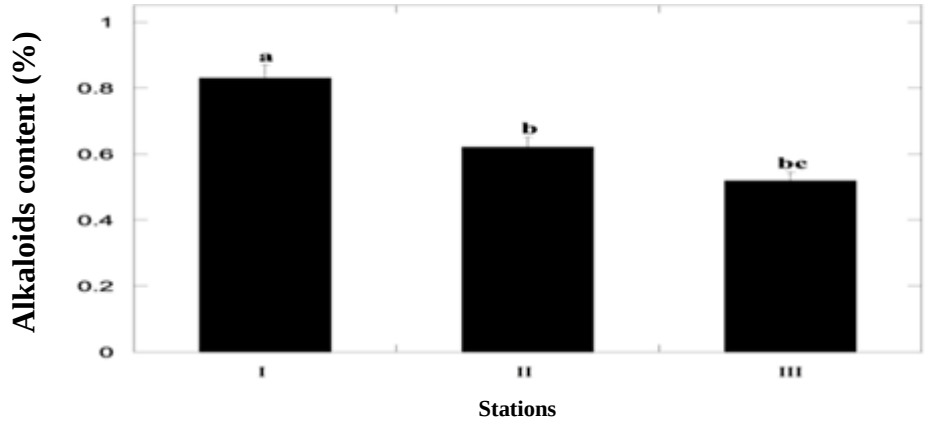

Figure 3. Histogram of alkaloid content research station of *Sarcophyton* sp. soft coral

Saponin Compound Content of *Sarcophyton* Sp. Soft Coral

The saponin content of *Sarcophyton* sp. soft coral was found varied also among research stations, i.e. 0.92% at station I, 0.74% at station II and 0.52% at station III. Based on the t-test results showed that the saponin content at station I was found significantly higher than that at stations II and III ($P< 0.05$). Similarly, saponin content at station II was found significantly higher ($P<0.05$) than that at station III. Therefore, environmental condition affects also saponin content in soft coral. Salinity is one of the limiting factors in coral growth. Low seawater salinity will make it difficult for coral animals to grow in these waters. The salinity level at station I is relatively lower, with an average value of 29o/oo, when compared to the salinity levels at station II, which has an average value of 30o/oo, and station III, which has an average value of 32o/oo. The normal salinity required by soft corals to carry out normal metabolism is in the range of 30-36 o/oo. Arafat (2009) stated that in areas with low salinity, such as river estuaries, corals are rarely found, and in areas with high rainfall, corals will experience disturbances, as will be the case in waters with very high salt content. This is because salinity can inhibit the growth rate of soft corals. The soft corals might be produced more saponin compounds at station I, presumably as a form of self- defense against environmental conditions that are not conducive to their survival. Dedi et al. (2017) said that changes in salinity can cause damage to important cells in the bodies of coral animals, thereby disrupting their development. Iswani et al. (2014) also added that soft corals in low salinity stress conditions will increase the production of secondary metabolites, one of which is saponin. The low salinity of the water at station I is thought to be the cause of increased saponin compound production at this station.

Saponin compounds are compounds that have a function in the reproduction process and also in the competition for space in soft corals. Pranoto, et al. (2012) stated that saponin compounds are a group of compounds that can inhibit or kill microbes by interacting with sterol membranes. The main effect of saponin on bacteria is the release of proteins and enzymes from bacterial cells. Saponins are produced as a form of chemical defense mechanism for soft corals. In addition to being suspected as a defense against predator attacks, saponin compounds are also believed to play a role in the reproductive process of soft corals (Nurjanah et al., 2011).

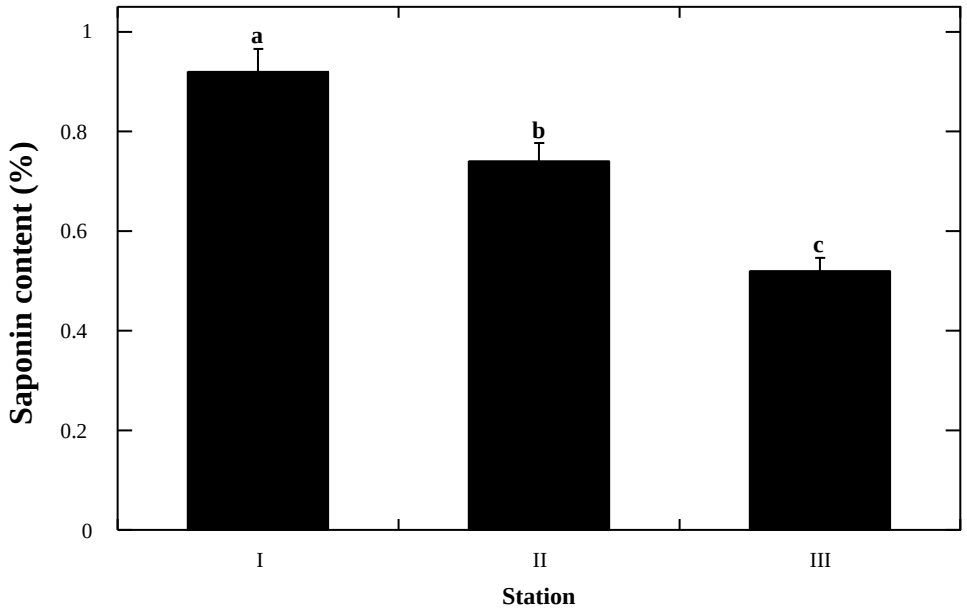

Figure 4. Histogram of saponin content (%) at each station of *Sarcophyton* sp. soft corals.

CONCLUSION

This study elucidated variation of secondary metabolic compound produced by soft corals of *Sarcophyton* sp in different water depth of Lara Island, Southeast Sulawesi. All measured compounds of tannin, flavonoid, alkaloid and saponin content in soft corals showed decreased trends as increasing in water depth. The content of secondary metabolites in *Sarcophyton* sp. in the successively stations of I, II and III were estimated as respective 0.54%, 0.37%, and 0.28% for tannins; 0.47%, 0.43%, and 0.34% for flavonoids; 0.81%, 0.64%, and 0.57% for alkaloids and 0.92%, 0.74%, and 0.52% for saponins. The contents of tannins, flavonoids, alkaloids, and saponins at Station I were significantly higher as compared to Stations II and III. This study revealed that environmental conditions, particularly salinity, play a crucial role in regulating the production of secondary metabolites in soft corals. Low-salinity environments impose osmotic stress, which can increase the accumulation of bioactive compounds as part of coral adaptation mechanisms. Therefore, environmental conditions play a very important role in producing secondary metabolite compounds, which support the soft corals to survive in extreme environmental conditions, especially low salinity stress.

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