

## Standardization of *Simplicia* Golden Sea Cucumber (*Stichopus hermanii*) from Pelapis Island, West Kalimantan

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### ABSTRACT

Standardization of medicinal ingredients and drug preparations is a requirement to ensure reproducibility of pharmaceutical and therapeutic quality. With standardization, it is hoped that it can further increase trust in medicines derived from natural ingredients. Golden sea cucumber is one of the natural ingredients that can be used as raw material for wound healing ointment preparations. The main content in golden sea cucumbers that play a role in wound healing is protein. This study aims to standardize the *Simplicia* of golden sea cucumber (*Stichopus hermanii*) originating from Pelapis Island, West Kalimantan. Standardization uses physical, chemical, and microbiological parameters like raw material requirements for standardized herbal medicines (OHT). The test is carried out in the laboratory using the method according to SNI. The results of the *Simplicia* test of golden sea cucumbers from Pelapis Island, West Kalimantan, included water content of 9.57%, zinc 9.32 mg/kg, cadmium < 0.030 mg/kg, copper < 0.030 mg/kg, lead < 0.066 mg/kg, mercury < 0.007 mg/kg, Total Plate Number 8.0 x 10<sup>3</sup> colonies/g, E.Coli < 3 APM/gr and Salmonella negative have fulfilled the requirements. The results of the *Simplicia* test of golden sea cucumbers include 13.4% ash content, 1.52% acid insoluble ash, 36.1% protein, 1.39% total fat, 13.1 mg/kg magnesium, and 10.6 mg iron. /kg. The results of the phytochemical screening of golden sea cucumber *Simplicia* were positive for containing alkaloids, saponins, and triterpenoids.

**Keywords** : standardization; *Simplicia*; golden sea cucumber; *Stichopus hermanii*; wound healing ointment

### INTRODUCTION

Standardization is an effort to improve product quality and safety. Standardization of drug ingredients and drug preparations is a requirement to ensure the reproducibility of pharmaceutical and therapeutic quality. With standardization, it is hoped that it can further increase trust in medicines derived from natural ingredients. Standardization of medicinal materials includes starting materials, intermediates, or finished products. One of the medicinal ingredients that are often used in natural medicinal preparations is *Simplicia*. Standardization of *Simplicia* needs to be done to get a repeatable effect (*reproducible*) (Wijaya, Setiawan, and Purnama, 2019).

The golden sea cucumber (*Stichopus hermanii*) is one of the marine animals classified as marine invertebrates belonging to the phylum Echinoderms (Pringgenies, Rudiyananti, and Yudiati, 2018). Sea cucumbers can be found in all coastal waters from shallow tidal areas to deeper waters. The main habitats of sea cucumbers are coral and seagrass beds. The distribution of sea cucumbers in Indonesia, among others, in East Java, Madura, Sumba, Bali, Aceh, Lombok, Bangka, Bengkulu,

Riau and surrounding areas, Belitung, Maluku, Papua, Sulawesi, Thousand Islands, and Kalimantan (west, east and south) (Leha *et al.*, 2020). Sea cucumbers contain protein and this protein has many benefits for the body. According to Rasyid (2018), sea cucumbers contain 86% protein that is easily soluble in the pepsin enzyme (Rasyid, 2018). Sea cucumbers, apart from being a source of protein, are also known to be used as medicine. Sea cucumbers have properties like anti-inflammatory, antioxidant, antiviral, antimicrobial, preventing blood clots/clotting, accelerating wound healing, preventing arthritis, high protein food source, anti-fatigue, boosting the immune system, and preventing cancer (Nurwidodo *et al.*, 2018). Sea cucumbers can regenerate themselves within 10-90 days so that they are whole again because sea cucumbers contain *cell growth factors* and high protein content. The ability of sea cucumbers to regenerate cells is the main basis that sea cucumbers can heal wounds. Therefore, sea cucumbers can be used as an ointment to heal wounds (Suryaningrum, 2008).

*Simplicia* of golden sea cucumber is a natural ingredient that can be used as a raw material for wound healing ointments. This study aims to standardize the *Simplicia* of golden sea cucumber (*Stichopus hermanii*) originating from Pelapis Island, West Kalimantan. Standardization

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uses physical, chemical, and microbiological parameters like raw material requirements for standardized herbal medicines (OHT).

## METHODOLOGY

### Materials

The tools used in making *Simplicia* are scales, containers, knives, boiling tools, drainers, smoking tools, cutting boards, bamboo winnows, and glass jars. Testing tools for water content, ash content, and acid insoluble ash content are blender, mesh No.20, desiccator, analytical balance, oven, porcelain crucible, furnace, and filter paper. Phytochemical screening test tools are analytical balance, test tube, stirring rod, filter paper, stainless spoon, measuring cup, beaker, water bath, and dropper. Protein content testing equipment includes a 100 ml Kjeldahl flask, a distiller and its accessories, as well as an electric heater or burner. The material used in the manufacture of *Simplicia* is a sample of fresh golden sea cucumber (*Stichopus hermanii*). The materials used in the test of water content, ash content, acid insoluble ash content, phytochemical screening, and protein content were golden sea cucumber *Simplicia* powder, 0.2 N hydrochloric acid (HCl), and aqua dest. The materials used in the phytochemical screening examination were hydrochloric acid, distilled water, Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, zinc, and amyl alcohol, 1% iron (III) chloride reagent, n-hexane, Libermann-Burchard, NaOH and benzene. The materials used in the protein content test were a mixture of selen (SeO<sub>2</sub>, K<sub>2</sub>SO<sub>4</sub>, CuSO<sub>4</sub>, 5H<sub>2</sub>O), mixed indicator (0.1% Bromocresol green solution and 0.1% methyl red solution), PP indicator, 2% boric acid H<sub>2</sub>BO<sub>3</sub> solution, a solution of 0.01 N HCl hydrochloric acid, and a 30% sodium hydroxide solution of NaOH.

### Methods

#### Sample collection

Golden sea cucumber (*Stichopus hermanii*) was obtained from marine waters in Pelas Island, Dusun Raya, RT 07, Pelapis Village, Karimata Islands District, North Kayong Regency, West Kalimantan.

#### Determination

Golden sea cucumber (*Stichopus hermanii*) was determined at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, West Kalimantan.

### Making simplicia

Fresh golden sea cucumbers that have been collected are carried out in wet sorting. The golden sea cucumber is cleaned by separating the internal organs and dirt from the body and then washing it with clean water. The golden sea cucumbers are then boiled and followed by smoking and drying in the sun (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). The dried golden sea cucumbers are finely chopped. The golden sea cucumber slices are dried in the sun to dry and break easily. The *Simplicia* dry sorting was then packaged and stored. For testing *Simplicia* with physical, chemical, and microbiological parameters, golden sea cucumber *Simplicia* powder was used. The powder was made by blending dried golden sea cucumber *Simplicia* and then sieved with mesh no.20.

### Organoleptic

Organoleptic test of the *Simplicia* of golden sea cucumbers including smell, taste, color, and shape using the five senses (Departemen Kesehatan RI, 2000).

### Parameters test

Water content, ash content, acid insoluble ash content, total phenol equivalent gallic acid, calcium (Ca), phosphorus (P), iron (Fe), alkaloids (qualitative), tannins (qualitative), total saponins from Quillaja bark Quantitative, total flavonoids, EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid) were carried out by sending samples to the Research Laboratory and Integrated Testing (LPPT) Gajah Mada University, Yogyakarta. Phytochemical screening was carried out by sending samples to the Pontianak State Polytechnic Chemistry Laboratory, West Kalimantan. Acid insoluble ash test, water content, ash content, protein, total fat, iron (Fe), cadmium (Cd), magnesium (Mg), zinc (Zn), copper (Cu), lead (Pb), mercury (Hg), *Escherichia coli* (E.coli), Total Plate Number (ALT), and *Salmonella* were carried out by sending samples to the Laboratory of Research and Industrial Standardization Institute (BARISTAND) Pontianak City, West Kalimantan.

## RESULT AND DISCUSSION

### Sample collection

Samples of golden sea cucumbers (*Stichopus hermanii*) were obtained from fishermen in Pelapis Village, Karimata Islands District, North Kayong Regency, West Kalimantan. The collection of sea cucumbers at Pelas was carried out in October

2021 because many sea cucumbers appeared there in October-November. This type of sea cucumber lives in reef areas and is rocky in these areas. The sea cucumbers obtained must have the form of fresh whole sea cucumbers and come from uncontaminated waters (2732:2-2009 Standard Nasional Indonesia (SNI), 2009). Sea cucumbers are taken at night because sea cucumbers are active at night and hide during the day. The sea cucumbers obtained from the sea are put into a container filled with saltwater or seawater until they reach the shore before being processed further so that the sea cucumbers are not destroyed.

### Determination

Determination of animals aims to identify and ensure the identity of the animals used, to avoid mistakes in taking. Based on the results of the determination conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) Tanjungpura University (UNTAN) stated that the animals used in this study were proven to be golden sea cucumbers (*Stichopus hermannii*).

### Making simplicia

Golden sea cucumbers that have been taken from the sea are processed by local fishermen. Processing is still done traditionally. The next step is wet sorting. The purpose of wet sorting is to separate good sea cucumbers and bad sea cucumbers. The sea cucumbers used must be clean, free from any odor that indicates spoilage, free from signs of decomposition and adulteration, and free from other natural properties that can degrade the quality and do not endanger health. Organoleptically, good sea cucumbers used have characteristics, namely intact appearance, clean and yellow color, smell of sea cucumber specifications, as well as dense, compact and elastic texture (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). The sea cucumbers that have been sorted wet are weighed. 6 sea cucumbers were taken with a weight of 1 kg and a length of 35 cm. Sea cucumbers are cleaned of dirt and entrails. The goal is to get clean sea cucumbers and reduce the contamination of pathogenic bacteria. Removal of the contents of the entrails aims to avoid the process of decay in sea cucumbers because the entrail contents contain microorganisms that can cause rot in sea cucumbers (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). In this study, the contents of the entrails were removed by stabbing the bottom with a knife and then removing the contents of the entrails. Furthermore, the golden sea cucumbers are washed clean. According to SNI

2732.3:2009, sea cucumbers are washed using cold running water. Washing is carried out on the outside and inside of the sea cucumber so that the remaining contents of the stomach are completely clean. Washing is carried out quickly, carefully, and in a sanitary manner (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). Sea cucumbers must be processed immediately when they reach the beach. When removed from the water, the sea cucumber's body will quickly soften and its body wall will drip (Ruppert, Fox, and Barnes, 1997). After cleaning, immediately boil the sea cucumbers until they harden. The purpose of boiling is to get a texture that meets specifications (hard, dense, compact, and tough), and to inactivate enzymes and microorganisms (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). By boiling it first, it will make the body of the sea cucumber harden and make it easier for the processing to be carried out to the next stage (Purcel, 2014). In this study, sea cucumbers were boiled for 2 hours with clean water. The sea cucumbers that have been boiled are then drained until not much water drips. Before drying, sea cucumbers are smoked first. In this study, smoking was carried out for 24 hours. According to SNI 2732.3:2009, fumigation aims to obtain smoked sea cucumbers with dryness levels according to specifications, namely water content of less than 20% (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). In this study, sea cucumbers were smoked over low heat smoldering. The sea cucumbers are placed far over the fire so that they are not exposed to direct heat. Drying is done on a para-para approximately 75-100 cm from the ground. Keep the fire not too hot (high heat), too hot will cause the sea cucumber skin to become too hard which will slow down drying. The fumigation of sea cucumbers is not carried out for a long time so it does not have a strong smoke smell (Purcel, 2014). After the fumigation is complete, the sea cucumbers are dried in the sun, the sea cucumbers are dried in the sun. The drying of sea cucumbers in this study was carried out for 4 days. The sea cucumbers are arranged evenly on the para-para and then dried in the sun. During drying, periodic reversal is carried out so that the sea cucumbers can dry evenly. According to SNI 2732.3:2009, sea cucumbers are dried to prevent mold growth when stored. The sea cucumbers are dried to get sea cucumbers with dryness according to specifications, namely a maximum moisture content of 20% and free from pathogenic bacteria (2732.3-2009 Standard Nasional Indonesia (SNI), 2009). Sea cucumbers are not dried in hot temperatures so that the skin is not tough and prevents the inside from getting wet or dry. The drying of sea cucumbers is carried

out in the sun. The ideal drying temperature should be between 35-45°C. Hotter temperatures for drying are not good because the body of the sea cucumber will roll or wavy because it shrinks too quickly (Purcel, 2014). The dried sea cucumbers were then weighed. The weight of the dried sea cucumbers was found to be 998 grams.

The sea cucumbers that have been processed by coating fishermen are then chopped into thin strips and cleaned of impurities that are still attached to the inner body walls of the sea cucumbers. Thin chopping is done to facilitate further processes such as drying, packaging, and storage. Chopping also aims to expand the surface to make it easier to dry (Mukhrani, 2014).

The chopped sea cucumbers are then dried again by drying in the sun. In this study, drying was carried out for 3 days. Drying is done to get Simplicia that is not easily damaged, so it can be stored for a longer time. Reducing the water content and stopping the enzymatic reaction, will prevent a decrease in quality or damage to Simplicia (Departemen Kesehatan Republik Indonesia, 1985). The sea cucumbers that have been dried again are subjected to dry sorting to separate the completely dry sea cucumbers, which are easy to break. The golden sea cucumber Simplicia was then weighed. Sea cucumbers are stored in a glass container, tightly closed, and protected from direct sunlight. Dried sea cucumbers must be stored in a dry room, protected from causes that can damage or reduce product quality such as moisture, insects, and rodents (2732.3-2009 Standard Nasional Indonesia (SNI), 2009).

### **Simplicia test results**

Simplicia golden sea cucumbers are light brown with a distinctive aroma and slightly salty taste. The water content indicates the number of free water contained in the Simplicia (2732.2-2009 Standard Nasional Indonesia (SNI), 2009).

The results of the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta can be seen in Table I. Based on the results of the Pontianak Industrial Research and Standardization Institute, showed that the water content of Simplicia golden sea cucumbers was 9.57%. According to SNI 2732. 1-2009, the maximum content for dry sea cucumbers is 20%. The water content of the golden sea cucumber Simplicia can be stated as low. Moisture content is related to the length of storage. Low water content can reduce the growth of microorganisms and inhibit enzymatic reactions so that Simplicia can be stored longer (Departemen Kesehatan RI, 2000). The ash content illustrates the mineral content

found in the golden sea cucumber Simplicia. The test results from the Pontianak Industrial Standardization and Research Institute showed that the ash content of Simplicia golden sea cucumbers was 13.4%. The higher the ash content produced by Simplicia, the higher the mineral content (Departemen Kesehatan RI, 2000). Acid insoluble ash content indicates the presence of metal or mineral contamination that is not soluble in acid in the Simplicia. The results of the acid-insoluble ash content test from the Pontianak Industrial Research and Standardization Institute, namely 1.52%. High acid insoluble ash content indicates the presence of silicate content from sand, soil, or metallic elements lead and mercury (Utami *et al.*, 2017). Sea cucumbers contain high protein which is good for wound healing by acting as a basic building block in the process of regeneration of body tissues (Suryaningrum, 2008). The results of the protein content test from the Pontianak Research and Industrial Standardization Institute showed that Simplicia golden sea cucumbers contained 36.1% protein. The protein content of sea cucumbers in wet conditions ranges from 2.5 to 13.8%/ww, while in dry conditions it ranges from 40.7 to 63.3%/wk (Wen, Hu, and Fan, 2010; Mehmet *et al.*, 2011). Total fat includes a group of compounds consisting of simple lipids, lipid compounds, and lipid derivatives that are soluble in chloroform, ether, or other organic solvents (Standarisasi Nasional Indonesia (SNI), 2017). The test results from the Pontianak Industrial Standardization and Research Institute, the total fat contained in golden sea cucumber Simplicia is 1.39%. This result is smaller than that obtained by Martoyo *et al.*, the total fat content in sea cucumbers is 1.7%. The fat contained in golden sea cucumber Simplicia is smaller than the average total fat possessed by sea cucumbers (Martoyo, Aji, and Winanto, 2002).

Sea cucumbers contain various kinds of minerals. The test results from the Pontianak Industrial Standardization and Research Institute can be seen in Table I, There are three minerals contained in golden sea cucumber Simplicia, namely iron (Fe), magnesium (Mg), and zinc (Zn). The content of zinc (Zn) in golden sea cucumber Simplicia is 9.32 mg/kg. Zinc is an essential cofactor in normal cell replication and growth and involves many enzymes in wound healing, such as DNA polymerase products. Zinc (Zn) is also widely involved in immune responses such as bacterial activity (Sari, Rima Parwati; Karsini Isidora; Rizal, M. Baroni; Tjhoeng, 2020). Magnesium (Mg) contained in golden sea cucumber Simplicia is 13.3 mg/kg. Magnesium can trigger VEGF which can accelerate the wound healing process to stimulate

Table I. Results of Simplicia test of golden sea cucumber (*Stichopus hermanii*) conducted at the Research and Industrial Standardization Center of Pontianak

Parameter	Unit	Test	Method
Moisture content	%	9.57	SNI 01 - 2891 - 1992
Ash content	%	13.4	SNI 01 - 2891 - 1992
Ash Insoluble in Acid	%	1.52	SNI 01 - 2891 - 1992
Protein	%	36.1	SNI 01 - 2891 - 1992
Total Fat	%	1.39	SNI 01 - 2891 - 1992
Magnesium (Mg)	mg/kg	13.3	SNI 01 - 2896 - 1998
Iron (Fe)	mg/kg	10.6	SNI 01 - 2896 - 1998
Zinc (Zn)	mg/kg	9.32	SNI 01 - 2896 - 1998
Cadmium (Cd)	mg/kg	< 0.030	SNI 01 - 2896 - 1998
Copper (Cu)	mg/kg	< 0.030	SNI 01 - 2896 - 1998
Lead (Pb)	mg/kg	< 0.066	SNI 01 - 2896 - 1998
Mercury (Hg)	mg/kg	< 0.007	SNI 01 - 2896 - 1998
Total Colony Plate Number	/g	8.0 x 10 <sup>3</sup>	SNI-ISO 7218: 2013, item 10.3.
Escherichia Coli (E. Coli)	APM/gr	< 3	SNI 01 - 2897 - 1992, item 3
Salmonella	Negative/25 gr	Negative	SNI 01 - 2897 - 1992, item 4

angiogenesis (Sari, Rima Parwati; Karsini Isidora; Rizal, M. Baroni; Tjhoeng, 2020). While the content of iron (Fe) contained in golden sea cucumber Simplicia is 10.6 mg/kg. Metal contaminants tested on golden sea cucumber Simplicia at the Pontianak Research and Industrial Standardization Institute included cadmium (Cd), Copper (Cu), Lead (Pb), and mercury (Hg). The chemical contamination levels include cadmium (Cd) < 0.030 mg/kg, copper (Cu) < 0.030 mg/kg, lead (Pb) < 0.066 mg/kg, and mercury (Hg) < 0.007 mg/kg. According to SNI 2732.1-2009, metal contamination limits for dried sea cucumbers are cadmium (Cd) a maximum of 1.0 mg/kg, mercury (Hg) a maximum of 0.5 mg/kg, and lead (Pb) a maximum of 1.0 mg/kg. kg, and copper (Cu) a maximum of 20.0 mg/kg (2732.1-2009 Standard Nasional Indonesia (SNI), 2009). These results show that the metals cadmium, lead, copper, and mercury which depend on the Simplicia of golden sea cucumbers still meet the requirements or are still acceptable. Microbial contamination tested on golden sea cucumber Simplicia at the Pontianak Industrial Standardization and Research Institute included Total Plate Number (ALT), *Salmonella*, and *Escherichia coli*. The results obtained include the Total Plate Number (ALT) of 8.0 x 10<sup>3</sup> colonies/g, *Escherichia coli* < 3 APM/gr and *Salmonella* negative according to SNI 2732.1-2009, the requirements for microbial contamination in dried sea cucumbers are a maximum Total Plate Number of 1.0 x 10<sup>5</sup>, *Escherichia coli* a maximum of < 3 APM/gr, and *Salmonella* negative (none at all) (2732.1-2009 Standard Nasional Indonesia (SNI), 2009). From these results, it can be said that the

microbial contamination contained in the golden sea cucumber Simplicia still meets the requirements or is acceptable.

Phytochemical screening of golden sea cucumber Simplicia was carried out at the Pontianak State Polytechnic Chemistry Laboratory, West Kalimantan. Phytochemical screening aims to qualitatively ensure the content of chemical compounds contained in golden sea cucumber Simplicia. Phytochemical screening was carried out in this study using a color test reaction and a foam test (for saponins). The results of phytochemical screening showed that the Simplicia of golden sea cucumbers from Pelapis Island, West Kalimantan, contained alkaloids, saponins, and triterpenoids. The alkaloid test of golden sea cucumber Simplicia powder with Dragendorff reagent gave a positive result in the form of an orange-brown precipitate. The saponin test on golden sea cucumber Simplicia powder by heating and shaking gave positive results in the form of foam formation. Steroid/triterpenoid test on golden sea cucumber Simplicia using Liebermann-Burchard reagent gave positive results in the form of red color. The red color formed indicates that the golden sea cucumber Simplicia powder is positive for triterpenoids.

The results of the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta can be seen in Table II.

Test results from the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta showed that the Simplicia of golden sea cucumbers contained saponins of 0.18 % w/w. Saponin compounds work as antimicrobials.

Table II. *Simplicia* test results for golden sea cucumber (*Stichopus hermanii*) based on Integrated Research and Testing Laboratory, Gadjah Mada University Yogyakarta

No.	Parameter Test	Result	Unit	Method
1.	Ash content	13.77	%	Gravimetry
2.	Moisture content	9.84	%	Gravimetry
3.	Acid Insoluble Ash content	0.20	%	Gravimetry
4.	Total Phenol Equivalent of Gallic Acid	1.51	% w/w	Spectrophotometry UV-vis
5.	Ca (Calcium)	16294.27	mg/kg	SSA-flame
6.	P (Phosphor)	523.96	mg/kg	Spectrophotometry UV-vis
7.	Fe (Iron)	58.64	mg/kg	SSA-flame
8-	Qualitative Alkaloids	Negative	-	TLC
9.	Qualitative Tannins	Negative	11.	TLC
10.	Total Saponins from Quillaja	0.18	% w/w	UV-vis Spectrophotometry
11.	Total Flavonoids	0.88	% w/w	UV-vis Spectrophotometry
12.	EPA ( <i>eicosapentaenoic acid</i> )	18.46	% Relative	Gas Chromatography
13.	DHA ( <i>docosahexaenoic acid</i> )	14.46	% Relative	Gas Chromatography

Saponins damage the cytoplasmic membrane and kill cells. The mechanism of action of saponins is to denature bacterial cell proteins and damage cell membranes without being able to repair them so that they can prevent infection in wounds. Flavonoids function by limiting the release of inflammatory mediators (Sari, Rima Parwati; Karsini Isidora; Rizal, M. Baroni; Tjhoeng, 2020). The test results from the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta showed that *Simplicia* golden sea cucumbers contained flavonoids of 0.88% w/w. The anti-inflammatory activity of flavonoids is by inhibiting cyclooxygenase and lipoxygenase, thereby limiting the number of inflammatory cells that migrate to the wound tissue. The activity of flavonoids in accelerating wound healing is also supported by an antioxidant mechanism that inhibits free radical activity. The high reactivity of flavonoid hydroxyl components causes free radicals to become inactive so that activity against inflammatory mediators by free radicals can be inhibited (Sari, Rima Parwati; Karsini Isidora; Rizal, M. Baroni; Tjhoeng, 2020). Phenolic compounds are known to play a role in preventing oxidation events. The total content of golden sea cucumber *Simplicia* phenolic compounds based on test results from the Integrated Research and Testing Laboratory, Gadjah Mada University Yogyakarta, was 1.51% w/w. The phenolic content in sea cucumbers is influenced by several factors such as the type of species, geography, physiology, and varying environmental conditions. Another factor that affects the presence of phenolic in sea cucumbers is food. The main source of food for sea cucumbers which are phenolic-rich materials, namely phytoplankton and particles from marine macroalgae, most likely affect the presence of

active phenolic compounds in the body wall of sea cucumbers. Sea cucumbers contain various kinds of minerals. The test results from the Integrated Research and Testing Laboratory of Gadjah Mada University Yogyakarta showed that the *Simplicia* golden sea cucumber contains minerals Calcium (Ca), Phosphorus (P), and Iron (Fe). The content of calcium (Ca) in golden sea cucumber *Simplicia* is 16294.27 mg/kg. Calcium regulates a large number of body physiologies, one of which is proliferation. The content of iron (Fe) in golden sea cucumber *Simplicia* is 58.64 mg/kg. The phosphorus (P) content in golden sea cucumber *Simplicia* is 523.96 mg/kg.

## CONCLUSION

The results of the determination of the sample indicate that the sample is confirmed to be a golden sea cucumber. The results of the *Simplicia* test of golden sea cucumbers include water content 9.57%, zinc 9.32 mg/kg, cadmium < 0.030 mg/kg, copper < 0.030 mg/kg, lead < 0.066 mg/kg, mercury < 0.007 mg/kg, ALT 8.0 x 10<sup>3</sup> colonies/g, E.Coli < 3 APM/gr and Salmonella negative met the requirements. The results of the *Simplicia* test of golden sea cucumbers include ash content 13.4%, acid insoluble ash 1.52%, protein 36.1%, total fat 1.39%, magnesium 13.1 mg/kg, and iron 10.6 mg./kg. The results of the phytochemical screening of golden sea cucumber *Simplicia* were positive for containing alkaloids, saponins, and triterpenoids.

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