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Standardization of Golden Sea Cucumber (*Stichopus hermanii*) Extracts from Pelapis Island, Kayong Regency, West Kalimantan

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Abstract

Stichopus hermanii can be used as a medicinal material, a source of animal protein, and wound healing medicine products. Extracts as raw materials for products must go through a standardization process to ensure pharmaceutical reproducibility, a therapeutic quality, and to ensure a consistent and uniform final composition. The purpose of this research was to determine the test results of the standardization parameters of *Stichopus hermanii* extract from Pelapis Island, West Kalimantan as raw material for wound healing herbal medicinal preparations. Preparation of extracts using the maceration method with 96% ethanol. The extract was standardized with specific parameters, including organoleptic, water and ethanol soluble content, phytochemical screening, and protein content, and non-specific parameters, including drying shrinkage, water content, ash content, and acid insoluble ash content. The test results obtained were a water-soluble content of 61.89%, ethanol-soluble content of 41.81%, protein content of 7.62%, drying shrinkage of 38.81%, water content of 20.58%, ash content of 37.95%, and acid insoluble ash content of 2.07%.

Keywords: golden sea cucumber; maceration; standardization; Stichopus hermanii.

INTRODUCTION

In this modern era, there is a lifestyle trend that leads to the use of natural ingredients as efficacious ingredients, both for medicine and body care products. This phenomenon has further increased the prestige of natural ingredients as an option because they are considered safer with lower negative effects (Nugroho, 2017). Since the last decade, the medical world's attention has begun to focus on marine biota as a very potential resource for producing active compounds (Bawole et al., 2021). Based on research by Roni et al, it is necessary to look for drugs based on marine biota, due to the increasing resistance of various diseases to existing types of drugs (Roni et al., 2020). Sea cucumber is one of the marine biota that may create bioactive substances that might be used as medicinal raw materials for medicines (Bawole et al., 2021).

More than 1400 species of sea cucumber have been identified in the world, among the identified species, *Stichopus hermanii* with its various contents has been empirically proven to be used as a wound medicine (D. W. Damaiyanti, 2015; W. Damaiyanti, 2015). Sea cucumber protein content reaches 82%, of all sea cucumber components, and 80% of the protein is collagen (D. W. Damaiyanti, 2015). The content of golden sea cucumber can be obtained by extraction methods, one of which is the maceration method using 96% ethanol which is then concentrated to obtain a thick extract. Extracts are made because the simplicia is no longer practical (Najib et al., 2018). The use of non-standardized extracts as herbal medicines can cause difficulties in quality control and the safety, efficacy, and quality profiles vary from one product to another (Syahidan & Wardhana, 2019).

Extracts standardization may be utilized to guarantee uniformity in the quality of raw materials, increase benefits, and ensure the safety and stability of extracts used to supporting health (Nurhaini et al., 2020). Based on Sumaryani, Taurina, and Andrie's research, characterization and standardization of *Stichopus hermanii* simplicia have been carried out and in Damaiyanti's research, characterization of water extracts in *Stichopus hermanii* has been carried out (D. W. Damaiyanti, 2015; Sumaryani et al., 2022; Taurina & Andrie, 2022). Research related to the standardization of golden sea cucumber extract has never been done. Based on the foregoing explanation, the researchers are interested in the standardizing *Stichopus hermanii* extract from Pelapis Island, North Kayong Regency, West Kalimantan as a raw material for herbal medicinal preparations with specific parameters, such as organoleptic, water–ethanol soluble essence content, phytochemical screening, and protein content, as well as

with non-specific parameters, such as drying shrinkage, moisture content, ash content, and acid–insoluble ash content.

METHODS

Samples and Materials

Samples of *Stichopus hermanii* simplicia were collected on Pelapis Island, West Kalimantan. The materials used were ethanol, aquadest, strong sulfuric acid *(H_2SO_4)*, *Mayer* reagent, *Dragendorff* reagent, *Wagner* reagent, Magnesium (Mg) metal band, hydrochloric acid (HCl), iron (III) chloride ($FeCl_3$), n–hexane, Liebermann-Burchard, gelatin, NaCl, K_2SO_4 *(Merck)*, $CuSO_4.5H_2O$ *(Merck)*, bromocresol green *(Merck)*, methyl red *(Merck)*, boric acid (H_3BO_3) *(Merck)*, and sodium hydroxide (NaOH) *(Merck)*. The *Stichopus hermanii* can be seen in Figure 1.

Figure 1. *Stichopus hermanii* (personal documentation).

Extract Preparation

The *Stichopus hermanii* dried powder obtained was then weighed up to 400 grams and then put into a maceration vessel and added ethanol 96% solvent in a ratio of 1: 4. During maceration, stirring was carried out as often as possible. The maceration process was carried out for 4x24 hours until the macerate became clear, then filtering and replacing new solvents every day after 24 hours of immersion. Then, all of the macerates were gathered and concentrated at a temperature 45˚C in a rotary evaporator till a thick extract was produced (Depkes RI, 2017).

Organoleptic Test

An organoleptic test is a testing technique that describes the qualities of a product's color, shape, sell, and taste utilizing the five senses (Depkes RI, 2000).

Water and Ethanol Soluble Essence Determination

Stichopus hermanii extract was weighed up to ± 2.5 g and then macerated for 24 hours with 50 mL of water–chloroform and ethanol using 2 different corked flasks. For the first six hours, the mixture was vigorously shaken. After being left for eighteen hours, it was filtered, with up to 25 mL of the filtrate being taken out, then evaporated in a waterbath to dry. The residue was heated to 105℃ till the weight persisted (Depkes RI, 2017).

Screening Phytochemical Determination

The identification of the alkaloids was carried out with 1 ml of HCl 2N and 9 ml of aquadest, they were then wheated in a waterbath for two minutes, cooled, and then filtered. The acquired filtrate was utilized for the alkaloid test and three test tubes were taken, then the filtrate obtained was placed into each test tube to which two drops of various reagents were added, namely Mayer, Dragendorff, and Wagner reagents (Nurjannah et al., 2022). Flavonoid identification is carried out by adding 3-4 little pieces of Mg metal tape and a few drops of strong HCl are added (Endarini, 2016; Yuliana et al., 2022). Phenol identification is done with 5-10 drops of $FeCl_3$ 3% (Yuliana et al., 2022). Identification of saponins is done with 10 ml of hot water and ferociously shaking motion for ten seconds. Characterized by the formation of a stable foam and will not disappear with the addition of single drop of HCl 2N (Yuliana et al., 2022). Identification of terpenoids/steroids is done with Liebermann-Burchard reagent (Fajriaty et al., 2018). Identification of tannins is done with $FeCl_3$ 3%. In addition, it is also done with 2 mL of gelatin 1% and a few drops of NaCl (Fajriaty et al., 2018).

Protein Content Determination

The *Stichopus hermanii* extract was weighed as much as ± 1 g of sample, then put into the Kjeldahl flask. Next, 3.5 g K_2SO_4 and 0.1 g $CuSO_4.5H_2O$, and 12 mL of concentrated H_2SO_4 were added and heated in a fume hood on an automatic digestion unit instrument, then cooled and 100 mL of distilled water was added. Installed the flask containing the results of the destruction in the distillation devices, then added 50 mL of 30% NaOH, then the distillate is collected in an erlenmeyer containing 30 mL of 4% H_3BO_4 which has been given an indicator, until the distillate droplets are neutral and the distillate is adjusted to 100 mL. Next, titrate the distillate with standardized 0.2 N HCl. A blank was made (Standar Nasional Indonesia (SNI) 01-2354.4-2006, 2006).

Drying Shrinkage Determination

The *Stichopus hermanii* extract was weighed up to 2 g, added, and tartered in porcelain crucible with a lid that had been preheated at 105°C for 30 minutes. The sample was leveled, placed in the oven, discovered, and dried at 105°C for 30 minutes or until the weight remained or until the discrepancy between the result of the two weighings was no greater than 0.5 mg (Depkes RI, 2017).

Water Content Determination
The *Stichopus hermanii* extract was weighed up to 2.5 g and placed in a weighing bottle with a cover whose weight was known. Then dried in the oven at 105°C for 3 hours. Following that, it was cooled in a desiccator and weighed (Depkes RI, 2017).

Ash Content Determination
The *Stichopus hermanii* extract weighed up to 2.5 g and was put into a porcelain cup that had previously been incinerated and weighed. The sample container was incinerated in a furnace at a steady weight of 600°C for 8 hours. After that, it cooled down into a desiccator, and then the cup containing the ash was weighed (Nurhidayah et al., 2019).

Acid Insoluble Ash Content Determination
The ash was boiled using 25 mL of diluted HCl 0.2 N for 5 minutes. The acid-insoluble portion was gathered, the solution was filtered through ash-free filter paper with a defined weight, and hot water washed the filter paper. The residue and filter paper were placed back into the silicated crucible and then burned in a furnace until a consistent weight was reached (Depkes RI, 2017).

RESULTS AND DISCUSSION

The *Stichopus hermanii* extract was created using the maceration procedure, which involves soaking in organic solvents for an extended period of time. The extract obtained was a thick brown extract of 51.40 grams with a yield of 12.85%. This shows the content and the extraction process is going well where the percent yield is more than 10%. the extract obtained was then measured for specific and non-specific parameters. The thick golden sea cucumber extract obtained after the evaporation process can be seen in Figure 2.

Figure 2. *Stichopus hermanii* Extract (personal documentation).

Organoleptic Test Result
Organoleptic parameters aim to provide a simple initial physical introduction to the extract that will be standardized using the five senses to describe the characteristics of the extract in the form of shape, color, and smell of golden sea cucumber extract.(Depkes RI, 2000) Organoleptic tests can determine the specific properties of an extract through direct observation based on general sources and can provide an overview of product damage and deterioration in the quality of ingredients during storage which can affect their properties (Evifania et al., 2020; Marpaung & Septiyani, 2020). The results of the organoleptic assay of *Stichopus hermanii* extract in this study are shown in Table 1.

Table 1. Organoleptic Test of *Stichopus hermanii* extract.

No.	Observations	Test Result
1.	Color	Brown
2.	Smell	The distinctive smell of sea cucumber
3.	Texture	Thick

Soluble Essence Content Result
The parameter of soluble essence content aims as a rough estimate of the presence and amount of bioactive compound content that is extracted in water solvents (polar) and ethanol solvents (semi polar – non polar) (Saifuddin et al., 2011). Determination of soluble essence content is very important because it can provide an overview of the number of dissolved ingredients and is part of what is utilized as a medicinal ingredient.(Zulharmitta et al., 2013) The results of the soluble essence content assay of *Stichopus hermanii* extract in this study are shown in Table 2.

Table 2. Soluble Essence Content of *Stichopus hermanii* Extract.

Parameters	Result	Methods
Water soluble essence content (%)	61,89	Gravimetr
Ethanol soluble essence content (%)	41,81	y

Based on the research of Nurhaini et al, the higher the percentage of juice content, the better the extract (Nurhaini et al., 2020). The results obtained show that the chemical compounds of golden sea cucumber extract are more easily dissolved and distilled in water than ethanol, which indicates that the content of polar compounds from *Stichopus hermanii* extract is more than the content of semi-polar-non-polar compounds.

Phytochemical Screening Result
Phytochemical screening is a way to qualitatively identify bioactive compounds in extracts (Manongko et al., 2020). Determination of phytochemical screening is carried out using a tube test consisting of precipitation reactions for alkaloid compounds, color reactions for phenol compounds, flavonoids, tannins, steroids and terpenoids, and foam formation for saponin compounds.

The results of the phytochemical screening assay of *Stichopus hermanii* extract in this study are shown in Table 3.

Table 3. Phytochemical Screening of *Stichopus hermanii* Extract.

No	Compound		Test Result
1.	Alkaloids	*Mayer*	(+) white precipitate
		Dragendorff	(–) no orange–brown precipitate formed
		Wagner	(+) brown precipitate
2.	Flavonoids	Mg + HCl	(+) orange color
		H_2SO_4	(+) red color
3.	Phenol	$FeCl_3$ 3%	(+) blackish-green color
4.	Saponins	Aquadest	(+) forms foam
5.	Terpenoids/Steroids	*Lieberman-Burchard*	(+) terpenoids (reddish color with reddish brown rings)
6.	Tannin	$FeCl_3$ 3%	(–) no intense green or blue color formed
		Gelatin 1%	(–) no white precipitate formed

The results of phytochemical screening tests show that *Stichopus hermanii* extract contains an alkaloid, flavonoid, phenol, saponin, and terpenoid compounds. These secondary metabolite compounds have bioactivities as antibacterials, antifungals, and antioxidants that can affect wound healing. The difference in the level of active compounds may be affected by the growth of a biota, both externally and internally. External factors include habitat, seasonality, water temperature, availability of food, and other environmental factors, while internal factors include age, body size, and other biological factors (Supriatna et al., 2019). These factors have potential to both qualitatively and quantitatively influence secondary metabolites, resulting in a wide range in both their content and bioactivity (Riwanti & Izazih, 2019).

Protein Content Result

The protein content parameter aims to determine the protein content contained in the extract (Afkar et al., 2020). The determination of total N was carried out to represent the amount of protein present because it is contained in all proteins which have a proportion of 16% of the total protein (Normilawati et al., 2019). The results of the protein content assay of *Stichopus hermanii* extract in this study are shown in Table 4.

Table 4. Protein Content of *Stichopus hermanii* Extract.

Parameter	Result	Methods
Protein content (%)	7,62	Kjeldahl

Based on Susanto's research, indicates that the composition of biological material varies depending on its geographic origin. Because of this, even though they are members of the same species, protein levels in golden sea cucumber vary from region to region (Susanto et al., 2018). Sea cucumbers high protein content helps speed up the regeneration of injured dead cells that help mend wounds. This is because of the presence of cell regeneration factors (also known as cell growth factors), are present, which may encourage regeneration to repair damaged cells or bodily tissues (Kokadir et al., 2021).

Drying Shrinkage Result

The drying shrinkage parameter seeks to set an upper bond (range) on the total amount of chemicals lost during drying as water and volatile substances (essential oils, ethanol solvents, or other substances) (Rosidah et al., 2020; Utami et al., 2017). For an extract to maintain quality and prevent mold formation (Sambode et al., 2022). The results of the drying shrinkage assay of *Stichopus hermanii* extract in this study are shown in Table 5.

Table 5. Drying Shrinkage of Stichopus hermanii Extract.

Parameter	Result	Methods
Drying shrinkage (%)	38,81	Gravimetry

The test results of drying shrinkage of *Stichopus hermanii* extract, amounted to 38.81% and can be said to not meet the general requirements of <10%. High drying shrinkage can also be caused by environmental factors such as low humidity which causes faster evaporation and consequently increases drying shrinkage. This can cause changes in the physical and chemical properties of the extract such as a decrease in volume and changes in the shape of the extract which can affect the quality and stability of the extract.

Water Content Result

The water content parameter set a minimum limit or range on the quantity of water contained that can be found in the extract and is used to determined how much water is left over after drying (Maryam et al., 2020; Najib et al., 2018). Determination of water content is not related to pharmacological activity directly but affects the quality, safety, and stability aspects of the extract and the formation of an extract preparation (Nurhaini et al.,

2020; Sambode et al., 2022). The results of the water content assay of *Stichopus hermanii* extract in this study are shown in Table 6.

Table 6. Water Content of *Stichopus hermanii* Extract.

Parameter	Result	Methods
Water content (%)	20,58	Gravimetry

Based on Damaiyanti's research, *Stichopus hermanii* extract from Bontang has a lower moisture content of 5.65% (D. W. Damaiyanti, 2015). The greater the percentage of water content in the extract, the easier it is for an extract to experience damage and decay caused by microbial growth. The high water content can be caused by a less than optimal drying process, the absorption of water into the extract caused by an environment that is too humid during the packaging and storage process, where sea cucumbers are hygroscopic because they have salt and collagen content that can absorb water (Kokadir et al., 2021; Suryaningrum, 2008).

Ash Content Result

The total ash content parameter is to provide an overview of the inorganic material and internal and external mineral contents from the initial process to extract production, in order to be related to the purity and contamination of an extract (Maryam et al., 2020; Zainab et al., 2016). The results of the ash content assay of *Stichopus hermanii* extract in this study are shown in Table 7.

Table 7. Ash Content of *Stichopus hermanii* Extract.

Parameter	Result	Methods
Ash content (%)	37,95	Gravimetry

The results of testing the ash content of *Stichopus hermanii* extract, which amounted to 37,95%, can be said that the sea cucumber extract does not meet the requirements of a maximum of 30%. High and low ash content can be caused by differences in organisms, habitat, living environment, and dietary factors (Elfath et al., 2019). Each water area can provide a different mineral intake. In addition, each organism also has a different ability to absorb minerals that enter the body, so this will affect the ash content value (One et al., 2021). High ash content can affect the effectiveness or biological activity of the extract, where these minerals can interact with active compounds in the extract so that they can change their physicochemical properties which affect changes in color, texture, or stability of the extract.

Acid Insoluble Ash Content Result

The acid insoluble ash content parameter aims to determine the levels of inorganic compounds and the presence of insoluble mineral or metallic contamination in acids such as silica from soil or sand, as well as silver, lead, and mercury metal elements (Utami et al., 2017). The results of the acid-insoluble ash content assay of *Stichopus hermanii* extract in this study can be seen in Table 8.

Table 8. Acid Insoluble Ash Content of *Stichopus hermanii* Extract.

Parameter	Result	Methods
Acid insoluble ash content (%)	2,07	Gravimetry

The test results of acid insoluble ash content of *Stichopus hermanii* extract, amounted to 2.07% and can be said to meet the general requirements of a maximum of 3.5% (Standar Nasional Indonesia (SNI) 01-2732-1992, 1992). High acid-insoluble ash content indicates residual contamination of acid-insoluble minerals or metals in the extract. The acid insoluble ash content can be used as a criterion to determine how clean a material is (Fitriyani et al., 2013). High level of acid insoluble ash also be an indication the presence of contaminants, such as heavy metal, which can change the composition and quality of the extract over time, reducing its effectiveness, stability and compromising its quality and safety.

CONCLUSIONS

The results of the specific parameter test of *Stichopus hermanii* extract from Pelapis Island consist of organoleptic examination which shows that golden sea cucumber extract has a brown color and typical sea cucumber odor, and is known to be positive for alkaloid, flavonoid, phenol, saponin, and terpenoid compounds. Golden sea cucumber extract has a water-soluble content of 61.89%, ethanol-soluble content of 41.81%, and protein content of 7.62%. While the test results of non-specific parameters of golden sea cucumber extract, consisting of drying shrinkage of 38.81%, moisture content of 20.58%, ash content of 37.95%, and acid insoluble ash content of 2.07%.

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