Antioxidant capacity of dry sea cucumber *Holothuria edulis*, *Pearsonothuria graeffei*, and *Stichopus herrmanni* from Boalemo waters, Gorontalo, Indonesia

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**Abstract.** Sea cucumber was a marine invertebrate from class Holothuroidea and phylum Echinoderm that possessed various bioactive compounds. This study aimed to estimate the antioxidant properties of sea cucumbers and evaluate their antioxidant ability. Ferric Reducing Antioxidant Power (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC) assay estimated the antioxidant properties of sea cucumbers, and for control, ascorbic acid and Trolox were used. The samples were dried sea cucumber of *Holothuria edulis*, *Pearsonothuria graeffei*, and *Stichopus herrmanni* from Boalemo, Gorontalo. The three samples had various antioxidant capacities. *H. edulis* had the highest value, both in FRAP and CUPRAC assay, followed by *P. graeffei* and *S. herrmanni*. The FRAP values were 48.47; 28.32; and 11.95 µmol Fe(II), respectively and the CUPRAC values were 6.5; 5.9; and 1.49 µmol Trolox/g extract. The weak antioxidant properties of sea cucumber extract in both methods were mostly related to the absence of phenolic compounds which usually have OH groups and conjugated double bonds. Separation and purification of the crude extract may improve its antioxidant properties. Based on this research, it can be concluded that the antioxidant capacity of *H. edulis*, *P. graeffei*, and *S. herrmanni* using the FRAP and CUPRAC method was weak.

**Keywords:** antioxidant capacity; CUPRAC; FRAP

1. **Introduction**

There are more than 400 species of sea cucumber in Indonesia, and the latest research results showed that 56 species were included in the types traded mainly for export purposes [1]. Sea cucumbers, a nutrient-rich marine invertebrate, have been used for centuries as an anti-inflammatory and anti-disease food source and to treat ailments in Korea, Japan, Indonesia, and China [2]. Sea cucumbers have received much attention in recent decades because of their nutritional value, health benefits, and potential for therapeutic use [3]. Bioactive secondary metabolites from sea cucumbers have been identified, including antiangiogenic, anticancer, anticoagulant, antihypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, and as a wound healing [4, 5]. Indonesian people use sea cucumbers as a food source, such as being processed into soup or processed into sea cucumber crackers, used to cure gout or joint pain [6-7]. Besides being pharmacologically useful, sea cucumbers also have the potential to be used as cosmetic ingredients. Sea cucumbers have been reported to have a high collagen content. This collagen is widely studied for its function to repair damaged tissue [8]. Research conducted on *Holothuria scabra* showed antioxidant activity [9], anti-wrinkle [10], and inhibition of melanogenesis [10]. Husni et al. [11] showed that the aqueous and ethanol extracts of *S. japonicus* had tyrosinase inhibitory activity. Based on this, it can be said that sea cucumbers are a potential source for application in health and cosmetics.

Antioxidants are substances that can inhibit oxidation and reduce the adverse effects of Reactive Oxygen Species [12]. The impact of oxidative stress can cause tissue damage, accelerated cell death,
and various diseases, such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders, heart disease, Alzheimer's disease, cognitive disorders, Parkinson's, and cancer. Many studies show that antioxidants play an essential role in maintaining human health and preventing and treating these diseases [13]. Recently, research has started to explore bioactive compounds that have the potential as antioxidants from the sea.

Several publications show that sea cucumbers contain compounds that have potential as antioxidants [9, 14, 15]. Although several studies have revealed the potential of sea cucumbers as a source of antioxidants, more information is needed to uncover and characterize the antioxidant potential of sea cucumber extracts. This study aims to determine the antioxidant activity of sea cucumbers Holothuria edulis, Pearsonothuria graeffei, and Stichopus herrmanni from the waters of Boalemo, Gorontalo through the Ferric Reducing Antioxidant Power (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC) assays.

2. Material and methods

2.1. Sample collection

Samples were obtained from the fisher’s village of Tilamuta, Boalemo Regency, Gorontalo Province, Indonesia. The samples used were sea cucumbers from the drying process carried out by fishermen in the village. The drying process involves washing, boiling, drying, and smoking. Identification of sea cucumber was carried out according to Patantis et al. [16].

2.2. Extraction

The sea cucumbers (100 g) were cut into 2-3 cm³ sizes, then the remaining sand and salt were cleaned. Extraction was carried out by maceration for 12 hours in methanol. The maceration process was carried out three times. The filtrate was filtered with Whatman filter paper. Then the methanol was evaporated using a vacuum rotary evaporator. The remaining methanol in the extract was then dried using a vacuum concentrator. The dry extract obtained was then calculated the yield with the formula:

\[
\text{Yield} = \frac{\text{extract weight}}{\text{dry weight sample}} \times 100\%
\]  

(1)

2.3. FRAP assay

The FRAP assay was conducted according to Kelman et al. [17]. FRAP reagent was prepared by mixing 300 millimolar (mM) acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) (Merck) solution and 20 mM FeCl₃·6H₂O in the ratio (10:1:1). Upon usage, the FRAP reagent was incubated at 37°C for less than one minute. The acetate buffer solution was prepared by diluting 3.1 g ram of sodium acetate trihydrate in 16 mL acetic acid glacial, then aquabidest was added up to the volume of 1 L. The TPTZ solution was prepared by diluting TPTZ in a 40 mM hydrochloric acid solution. In this analysis, FRAP reagent of 150 µL was poured into a 96-well microplate and added with seaweed extract (1 mg/ml dose) for 20 µL. The microplate was incubated in the darkroom of 27-28°C for 8 minutes. Then its absorbance was measured in a microplate reader (Thermo Scientific) at the wavelength of 595 nm. The Ferrous (Fe²⁺) standard curve was constructed simultaneously at FeSO₄· 7H₂O concentration range of 50 - 1000 µM. FRAP value in µmol Fe(II) was determined using the equation formulated from the standard curve.

2.4. CUPRAC assay

The CUPRAC antioxidant test procedure refers to Ardhani et al. [18] with minor modifications. A total of 50 L CuCl₂·2H₂O 0.01 M, 50 µL neucuproine (Merck) 7.5 x 10⁻³ M, 50 µL ammonium acetate buffer pH 7, and 50 µL sample solution with a concentration of 625; 1250; 2500; and 5000 µg/ml was added to a 96-well microplate. Standard curves for measuring antioxidant capacity using Trolox (Merck) with
concentrations of 20, 30, 40, and 50 µM. The buffer solution was used as a blank. The mixture in the microplate was incubated for 30 minutes, and the absorbance was measured using a microplate reader at 450 nm.

2.5. Data analysis
An antioxidant test was carried out with three replications. Data is presented in the form of mean ± standard deviation. To find out whether there are differences in yield and antioxidant capacity between 3 species of sea cucumber, one-way ANOVA analysis was performed. Statistical analysis was carried out with the MINITAB 16.0 statistical program.

3. Result and discussion
Three types of sea cucumbers (H. edulis, P. graeffei, and S. herrmanni) were extracted in this study using methanol. Methanol was used because it had a strong affinity for extracting organic compounds in a broad polarity range. Non-polar fatty acid compounds, steroids, and polar saponins will be extracted well by methanol. S. herrmanni had the highest yield (9.50%), followed by P. graeffei (7.66%) and H. edulis (4.50%) (table 1). Methanol is the optimal solvent for extracting high yields of phytochemical constituents [19].

H. edulis has the local name "cera merah", "dada", "cera", "perut", "lakling merah", "talking", "batu keeling" and the common name for this sea cucumber is pinkfish [16, 1] (Fig 1). This sea cucumber has a low price in the market. P. graeffei is also known as cera duri, bintik merah, goomyok, sutra, gemuk, bati, donga [16, 1], and the common name for this sea cucumber is blackspotted sea cucumber and flowerfish. S. herrmanni was formerly known as S. variegatus. Sea cucumber S. monotuberculatus has also been reported as S. variegatus. S. herrmanni has the trade name curryfish and is locally known as Kasur [16], gamet mas, gamet kacang, and taikongkong [1]. In contrast to H. edulis and P. graeffei, S. herrmanni belongs to sea cucumbers with high economic value.

The results of the FRAP assay showed that H. edulis had the most potent antioxidant capacity compared to the others (p < 0.05). The antioxidant capacity of the FRAP method of H. edulis, P. graeffei, and S. herrmanni was 59.95, respectively; 25.50 and 11.80 µmol Fe(II)/g extract. The antioxidant capacity of the CUPRAC method also showed that H. edulis had the highest value (9.56 µmol Trolox equivalent/g), but it was not significantly different with P. graeffei (p > 0.05). The antioxidant capacity of the CUPRAC method with the smallest value was S. herrmanni. The antioxidant capacity of the three

Figure 1. Sea cucumbers were used in the study. Top: fresh or live sea cucumbers, bottom: dried sea cucumbers, in bottles: sea cucumber methanol extract.
sea cucumbers when compared with ascorbic acid (positive control) was low. At a much lower concentration (20 µg/ml), ascorbic acid has a FRAP value of 205.50 mol Fe(II)/g.

Murniasih et al. [20] tested the antioxidant activity of four species of sea cucumbers, namely *H. scabra*, *H. atra*, *H. leucospilota*, and *H. excellens*, using the DPPH method. The test results showed that *H. leucospilota* had the best antioxidant activity of 11.12% at a dose of 1.0 mg/ml extract. Other research showed that collagen hydrolysate of *S. horrens* had an IC$_{50}$ value of 5.25 mg/ml to DPPH free radicals [21].

FRAP was chosen because it measures the total combined activity of the active redox antioxidants; the reagent is inexpensive, low toxicity and very stable; clear procedures; results can be obtained within minutes; susceptible and precise; constant stoichiometric factor; and the test has been widely used and has been validated. Similar to FRAP, CUPRAC method was chosen because of its fast kinetics; using neutral pH (pH 7.0); more stable redox potential; the reagent is durable more stable and easier to obtain; versatile because it can test hydrophilic and lipophilic antioxidants; sensitive and has a linear response; low prooxidative potential; can be processed into other analytical techniques; and can be applied to food and medicine [13].

As mentioned above, compared with ascorbic acid as a positive control, the antioxidant capacity of the three types of sea cucumbers was weak. This condition is most likely related to the lack of aromatic ring compounds and hydroxyl groups, which play a significant role in their antioxidant properties. It is known that the primary compounds in sea cucumbers are saponins [22-23]. This sea cucumber is different, for example, with the content of antioxidant compounds found in brown seaweed. Brown seaweed contains a lot of phenolic compounds (for example, phlorotannin) with potent antioxidant properties and fucoxanthin pigments.

### 4. Conclusion

The results of the study using the FRAP and CUPRAC methods showed that the extracts of *H. edulis*, *P. graeffei*, and *S. herrmanni* had weak antioxidant capacity. Thus, it can be concluded that the 3 species of sea cucumbers have weak antioxidant properties. Other research is needed to reveal the potential of these sea cucumbers for cosmetics, for example, their collagen, peptide, or polysaccharide properties.

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### Table 1. Yield and antioxidant capacity of several species of sea cucumbers from Boalemo Waters, Gorontalo.

| Species Name          | Local Name | Yield (%) | FRAP µmol Fe (II)/g | CUPRAC µmol Trolox/g |
|-----------------------|------------|-----------|---------------------|---------------------|
| *Holothuria edulis*   | Cera Merah | 4.50°     | 46.95± 1.08         | 9.56±1.08           |
| *Pearsonothuria graeffei* | Cera Duri | 7.66°     | 25.50± 2.0          | 9.29±1.3           |
| *Sticopus herrmanni*  | Gamat      | 9.50°     | 11.80±0.1           | 2.32±0.8           |
| Positive control (Ascorbic acid) at 20 µg/ml |           |           | 205.50± 16.2 | |

Note: Different letters at the same column show significantly different (p<0.05)
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