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Phytochemicals analysis, and inhibitor enzyme a-glucosidase activity of teripang (*Stichopus hermanii*)

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Abstract
Isolation, purification and identification of the ethanol extract of sea cucumber (*Stichopus hermanii*) originating from the area of Seira Island, Ambon Indonesia has been done. Extraction was carried out by maceration of dried sea cucumbers with 96% ethanol (after cleaning, drying, smoked, and cut into small pieces). Ethanol extract was fractionated by column chromatography (SiO_2, $CHCl_3$ - MeOH = 10: 1 ~ 2: 1) gave 4 fractions (Fr. I ~ Fr. IV). GC-MS analysis for fraction III which resulting in □-glucosidase enzyme inhibition of 131.52 ppm consisting of compounds fatty acids and monosacharides.

Keywords: *Stichopus hermanii*; inhibition of enzyme □-glucosidase; anti-diabetic; Seira Island-Ambon

Introduction
Indonesia is an archipelago that has a diversity of flora and fauna. The Maluku Islands is an area that has a sea area larger than land, which causes people's livelihoods to focus more on the sea. Seira Island is in the administrative area of the Tanimbar islands, Wermaktian District, Tanimbar Islands Regency. Ambon. Seira Island is famous for its marine products such as fish, seaweed, seashells, sea cucumbers, and other marine products which can be used as a source of income and fulfill the consumption needs of the local community (Leha M.A *et al* 2020) [11]
Sea cucumbers are the leading commodity of the island, so the flow of sea cucumber buying and selling is very smooth, where buyers can go directly to the processing location, the selling price of sea cucumbers varies, ranging from tens of thousands of rupiah to millions of rupiah per kilogram according to their type and size. The sea cucumber, *Stichopus hermanii*, has a lower selling price compared to other types of sea cucumber, such as *Holoturia sraba*
Stichopus hermanii has different local names, local people call it Namat spots, while others are Gamete mas, Taikongkong, its trade name is curry fish (Setyastuti A, 2015). Sea cucumbers have active chemical substances that are useful in the pharmaceutical and health fields as a source of active ingredients as antibacterial, anti-fungal, anti-tumor and anti-coagulant (anti-clotting) (Farouk, *et al*, 2007). Zancan *et al* reported that sea cucumbers can be used for wound healing, and contain anticoagulant and antithrombotic compounds (Zancan, *et al*, 2004) [16], as antibacterial (Afiyatullov, 2002) [1], anti-tumor, anti-fungal, antibacterial and antiviral (Han Hua, *et al*, 2009) [7].
Various advantages and uses of sea cucumbers cause the price of sea cucumbers to be very high in the local and international market. The technology of *Stichopus hermanii* sea cucumber cultivation has been carried out to anticipate a decrease in the number of sea cucumber populations in the wild (Tatalede P.A *et al*, 2018; Budikasel A, *et al*, 2019, Tarimakase. Y, 2020) [15, 3, 14]. In connection with the benefits and abundance of sea cucumbers in Seira Island, Ambon, a study was conducted with the aim of knowing the chemical compound content and testing the enzyme □-glucosidase inhibitory activity against sea cucumber *Stichipus hermanii.*

Research Methods
Locations
Sampling and preparation of Sea cucumber (*Stichipus hermanii*) located in Seira island, Ambon, Indonesia (Fig 1)

Sample Preparation
Sea cucumbers are cleaned *in situ* using clean sea water to remove innards and dirt. Then the sea cucumbers are boiled until the meat is compact and hardened, the remaining contents of the stomach and offal are removed. The sea cucumbers are then boiled and then, smoked and dried in the sun and cut into small pieces.

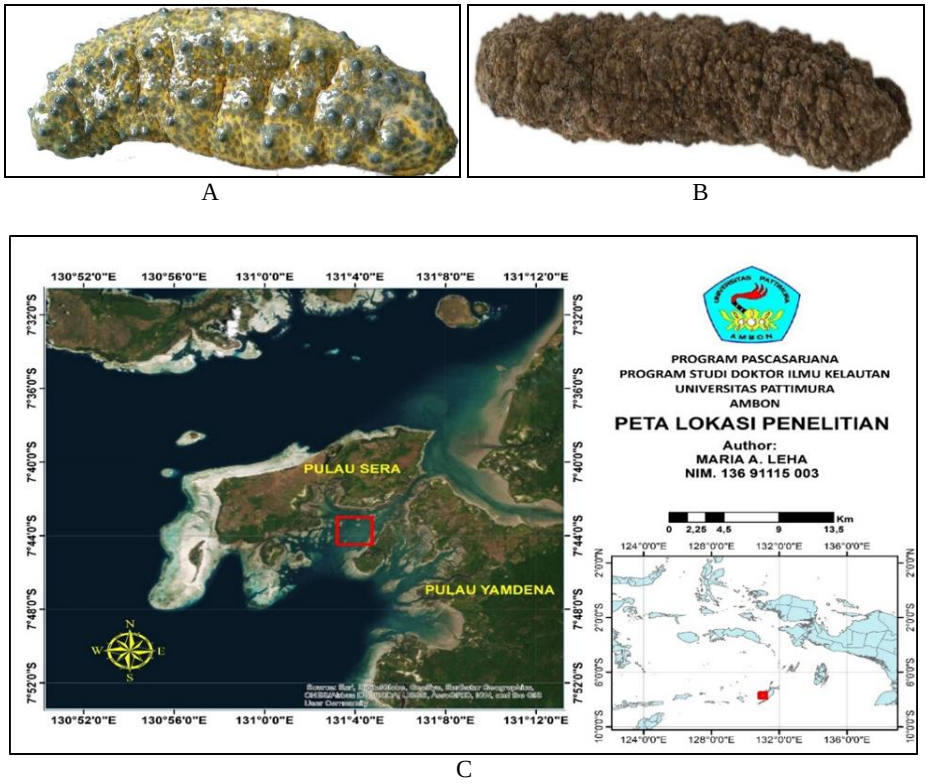

Fig 1: Sea cucumber (*Stichopus hermanii*) collected from Seira island, Ambon, Indonesia

a. Fresh Sea cucumber B. Dried Sea Cucumber C. Sample collection location (Seira Island, Ambon, Indonesia)

Extraction and fractionation

Some pieces of sea cucumber (100 g) extracted by maceration using ethanol 96% for 24 hours (repeated 6 times), filtered and evaporated until a dry extract (3.96 g) was obtained.

The ethanol extract (1.5 g) was processed with column chromatography (SiO_2; $CHCl_3$-MeOH = 10: 1 ~ 2: 1) gave 4 fractions. Then the four fractions were tested for inhibition of □-glucosidase enzyme. Furthermore, fraction 3 gave the best of □-glucosidase enzyme inhibitory activity. (Table 1). The scheme of sea cucumber extraction and fractionation can be seen in Figure 2

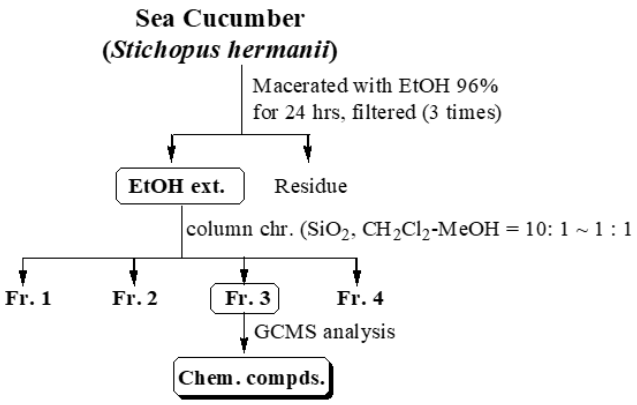

Fig 2: Isolation scheme, and fractionation of Sea cucumber (*Stichopus hermanii*)

□-glucosidase enzyme inhibition on the extract and the fractions of column chromatography

Test of □-glucosidase enzyme inhibition proceed using modified Molyneux method (Molyneux *et al*., 2002)

Phytochemical screening

Phytochemical screening for alkaloid compounds, flavonoid, steroid/terpenoid carried out based on Harborne method (Harborne, *et al*.1998)

Chemical compound analysis with GC-MS

GC-MS analysis was carried out on a Shimadzu GC-2010 plus series, and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column RTX-5-MS fused silica capillary column (30mm×0.15mm ID ×1 µMdf), operating in electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

Identification of Components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Results

The result of sea cucumber (*S. hermanii*) extraction by maceration obtained 3.96 g of ethanol extract with a yield of 3.96%. And the results of phytochemical screening gave a positive reaction to flavonoids, steroids/terpenoids and saponins. Isolation and fractionation by column

chromatography (SiO_2; CH_2Cl_2-MeOH = 10: 1 ~ 1: 1 gave 4 fractions and the results of the □-glucosidase enzyme inhibition test can be seen in Table 1

Table 1: Result of inhibition enzyme □-glucosidase and antioxidant assay for EtOH extract, and fractionation of column chromatography

No	Sample	Inhibition of enzym □-glucosidase activity (IC_{50}, ppm)
1	EtOH Ext.	**26.47**
2	Fr. 1	135.86
3	Fr. 2	356.64
4	Fr. 3	**131.52**
5	Fr. 4	236.46
6	Acarbose	17.72

The results of ethanol extract and column chromatography fractionation showed that there was a decrease in the inhibitory activity of the a-glucosidase enzyme from the ethanol extract to its fractions from IC_{50} (131.52 ~ 236.46 ppm) to IC_{50} 26.47 ppm (Table 1). which means that the chemical compounds in sea cucumbers are synergistic.

Ethanol extract of *Stichopus hermanii* sea cucumber contains active compounds, namely flavonoids, so that it is thought to have inhibitory activity of diabetes activity. This was also conveyed by Taufiqurohman (2015) that the active compounds in the extract, namely flavonoids, have inhibitory activity against α-glucosidase enzymes through the formation of hydroxylation bonds that are substituted on benzene rings. The principle of this inhibition is similar to the acarbose which is commonly used for diabetics to control blood sugar levels. Acarbose as an antihyperglycemic agent works competitively to inhibit reversible α-amylase pancreatic and membrane binding to the α-glucosidase enzyme in the intestine (Bayer, 2011).

Yuefei *et al.* (2012); Luo, *et al*. 2012; and Pujiyanto, *et al*. 2015 in their research reported that acarbose can be used as a comparison in testing α-glucosidase enzyme activity.

Chemical compounds using GCMS

Eight compounds were identified in Tripang (*Stichopus hermanii*) by GC-MS analysis. The active principles with their Retention time (RT), and Concentration (%) are presented in (Table 2 and Fig 2). The prevailing compounds were □-D-Glucopyranose, 1,6-anhydro-; L-Glucose, 6-deoxy-3-O-methyl.

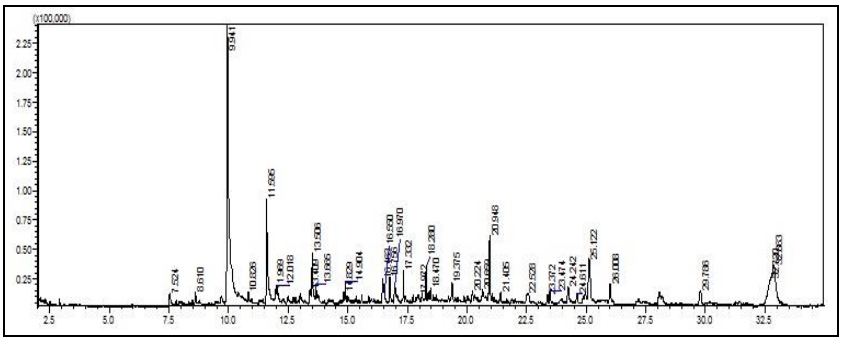

Fig 2: compound identification performed on III fraction of S. *hermanii* ethanol extract

Table 2: Fraction 3 of *S. hermanii* ethanol extract

No	RT	Area (%)	Name of compds	Biological activities
1	12.02	1.03	Octanoic acid	Therapy on Essential Tremors, Essential Voice Tremors and Obeticholic Acid (OCA) in Primary Sclerosing Cholangitis (PSC)
2	13.67	0.63	2-Decenal, (E)-	food flavoring
3	16.46	2.50	L-Glucose, 6-deoxy-3-O-methyl-	antidiabetic
4	16.97	1.65	□-D-Glucopyranose, 1,6-anhydro-	Antidiabetic
5	20.22	0.67	2-Tridecenal, (E)-	food flavoring
6	23.47	0.87	Hexadecenoic acid, methyl ester	Antibacterial, antifungal, Antioxidant, hypocholesterolemic, flavor, hemolytic 5-alpha reductase inhibitor, antiandrogenic
7	24.24	1.10	n-Hexadecenoic acid	Anti-inflammtory, hemolytic 5-alpha redustase inhibitor, antioxidant, hypocholes terolemic, Antioxidant, hypoolesterolemic anti androgenic, Anti-oxdant, anti-bacteria, anti-fungal and cosmetic. Inhibits HIV-1 infection (reduces the ability of the HIV-1 virus to enter CD4 + T-cells)
8	29.79	1.61	Methyl stearate	Food flavoring

Conclusion

The results showed that ethanol 96% extract of sea cucumber (*Stichopus hermanii*) had activity as an inhibitor of enzym □-glucosidase of IC_{50} 26.47 ppm) and decreased in the form of the third fraction (IC_{50} 151.32 ppm), which means that the chemical compounds in sea cucumbers are synergistic.

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