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Identification of Saponins from the Active Fraction Sea Cucumbers (*Stichopus vastus* sluiter)

Identifikasi Saponin dari Fraksi Aktif Teripang Kasur (Stichopus vastus sluiter)

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Abstract

This study aims to determine the secondary metabolic content of saponin contained in the active fraction of sea cucumber (*Stichopus vastus*) by using metabolic test reagents and thin-layer chromatography (TLC) analysis. The active fraction was given the reagent in the form of HCl 2N and strong acid then continued with analysis to determine the Rf value and lieberman-burchad reaction. The results of the metabolic reaction test showed that the active fraction contained steroidal saponin compounds characterized by the presence of a stable foam and the formation of a greenish-green color. TLC analysis produced a single stain with an Rf value of 0.9 and a bluish color reaction after being given Liberman-burchad which confirmed that the saponins contained in the active fraction of sea cucumbers were steroidal saponin.

Keywords: Sea Cucumber, *Stichopus vastus sluiter*, Secondary Metabolites Saponin

Abstrak

Penelitian ini bertujuan untuk mengetahui kandungan metabolik sekunder saponin yang terdapat pada fraksi aktif teripang kasur (*Stichopus vastus*) dengan metode reaksi (*reagen*) uji metabolik dan analisis kromatografi lapis tipis (KLT). Fraksi aktif diberi pereaksi berupa HCl 2N dan asam kuat kemudian dilanjutkan dengan analisis untuk penentuan nilai *Rf* dan reaksi *lieberman-burchad*. Hasil uji reaksi metabolik menunjukkan fraksi aktif mengandung senyawa saponin steroid ditandai dengan adanya busa yang stabil dan terbentuknya warna hijau kehijauan. Analisis KLT menghasilkan noda tunggal dengan nilai *Rf* 0.9 dan reaksi warna kebiruan setelah diberi *liberman-burchad* yang menegaskan senyawa saponin yang terkandung pada fraksi aktif teripang kasur merupakan saponin steroid.

Kata kunci : Teripang Kasur, *Stichopus Vastus Sluitter*, Metabolik Sekunder Saponin

1. Introduction

Sea cucumbers are marine biota from the echinoderm phylum that are spineless and soft-bodied, known as sea cucumbers or sea cucumbers due to their physical shape resembling a cucumber which is symmetrical with five hemispheres of the fingers (pentamerous radial symmetry). Sea cucumbers are included in the high economic marine biota and are an export commodity. According to Pranoto *et al.* (2012), Sea cucumbers contain bioactive or secondary metabolic compounds that can be used as functional food including alkaloids, saponins, triterpenoids, flavonoids, and steroids. The secondary metabolites of an organism are adapted to environmental conditions to defend themselves from environmental conditions. According to Raharjo (2013), organisms interact with their environment use secondary metabolites. Secondary metabolite compounds contained in sea cucumbers can be used to improve human health through processing and extraction processes.

Janakiram *et al.* (2015) stated that sea cucumber extract acts as an antioxidant that can inhibit free radicals and can prevent several degenerative diseases such as heart disease and cancer. To obtain metabolic compounds, whole sea cucumbers are extracted and fractionated using certain types of solvents. Lapornik *et al.* (2005) stated that methanol solvent was able to extract components derived from alkaloid, phenolic, steroid, triterpenoid, tannin, and saponin. The content of secondary metabolic compounds can be determined by qualitatively testing metabolic compounds using reagents. Secondary metabolic compounds will produce reactions when given the appropriate reagent such as alkaloids will produce white lumps when given the Mayer reagent. Flavonoids show a red/orange color when reacted with Mg and concentrated HCl. Saponin will produce foam when shaken. Steroids produce a bluish-green color and terpenoids will form a brown ring when chloroform, acetic acid, and sulfuric acid are added (Harborne, 1998). There are two kinds of saponins, namely, steroids (tetracyclic triterpenoids and pentacyclic triterpenoids). Both have a glycoside bond at C-3 and have biogenesis origins through mevalonic acid and isoprenoid (Evans, 2002)

2. Material and Method

2.1. Materials

The main ingredients of this research were hexane, ethyl acetate, and butanol fractions from sea cucumber (*Stichopus vastus* Sluiter) which were produced from the fractionation process. 2N HCl solution, aquadest, lieberman-burchad, butanol, acetic acid, and GF_{254} silica plate. The equipment used is a pencil, beaker glass, test tube, dropper pipette, capillary tube, analytical balance, 254-366nm UV lamp, and ruler.

2.2. Procedure

3 mg of the hexane, ethyl acetate, and butanol fractions were put into a test tube and 10 mL of distilled water was added and shaken for 10 seconds until foam formed. After the foam was formed, 5 drops of 2N HCl were added to stabilize the foam. To determine the type of saponin, the hexane, ethyl acetate, and butanol viscous fractions were put into each test tube as much as 3 mg, added 0.5 mL of chloroform and 0.5 mL of anhydrous acetic acid then added 1-2 mL of concentrated H_2SO_4, observe the resulting reaction. TLC analysis used a 60 GF_{254} silica plate measuring 10x5cm which had been heated in an oven at 100^oC for 30 minutes. The eluent used as the mobile phase was butanol: acetic acid: aquadest (4:1:5), the sample in the form of the fraction was dripped onto a silica plate and then put into a chamber containing the eluent. Observe the reaction and the resulting stain is then given Lieberman-Burchad reagent. Single stains were observed under a 254-366nm UV lamp.

3. Result and Discussion

3.1 Metabolic Compound Test and Thin Layer Chromatography Analysis

The metabolic compound test showed that there was a saponin compound in the n-hexane and ethyl acetate extract that was characterized by the formation of stable foam for 1 minute and then given 2N HCl the foam remained stable for 10 minutes. In the butanol extract, the foam was unstable and disappeared, so it was stated that there was no content or was negative for saponin compounds, so TLC analysis was not carried out. Saponins are active components with soap-like properties that produce foam when shaken with water (Robinson, 1995). Based on the test results, steroid compounds were detected positively in the n-hexane and ethyl acetate fractions. A positive steroid test result is indicated by the formation of a bluish-green color. No formation of brown or violet rings indicates that there is no terpenoid content in the n-hexane, ethyl acetate, and butanol extracts. Based on the sapogenin structure, there are two types of saponins, namely steroids, and triterpenoids, both of which have a C-3 glycosidic bond and have their origin in biogenesis through mevalonate acid and isoprenoids (Evans, 2002). Harborne (1998) states, steroid compounds will produce a blue or green reaction and terpenoids will produce a red or purple reaction when given a strong acidic reagent. The results of the secondary metabolic compound test can be seen in Table 1. Steroid compounds will produce a blue or green reaction and terpenoids will produce a red or purple reaction if given a strong acidic reagent.

Table 1. Thin Layer Chromatography Analysis Results

Fraction	Rf value	*Lieberman*Burchad		Information
		Visual	UV 254	
Hexane	0.90	Yellow	bluish	(+) Steroid Saponins
Ethyl Acetate	0.91	Yellow	bluish	(+) Steroid Saponins

Thin layer chromatography (TLC) analysis with the eluent used as the mobile phase, namely, Butanol: acetic acid: aquadest (4:5:1). The eluent dilutes the compound to produce spots or stains which will be used in determining the Rf value. The resulting stain is visible visually and UV lamp 254-366. On the TLC plate, a single stain (no tail) is visible, so it can be said that the compound has been isolated. According to Markham (1988), a well-isolated compound will produce a tailless stain and no other stains will form along the eluent so that the separation of the stains is clear. The results of the TLC analysis of the hexane and ethyl acetate fractions and the resulting stains can be seen in Figure 1.

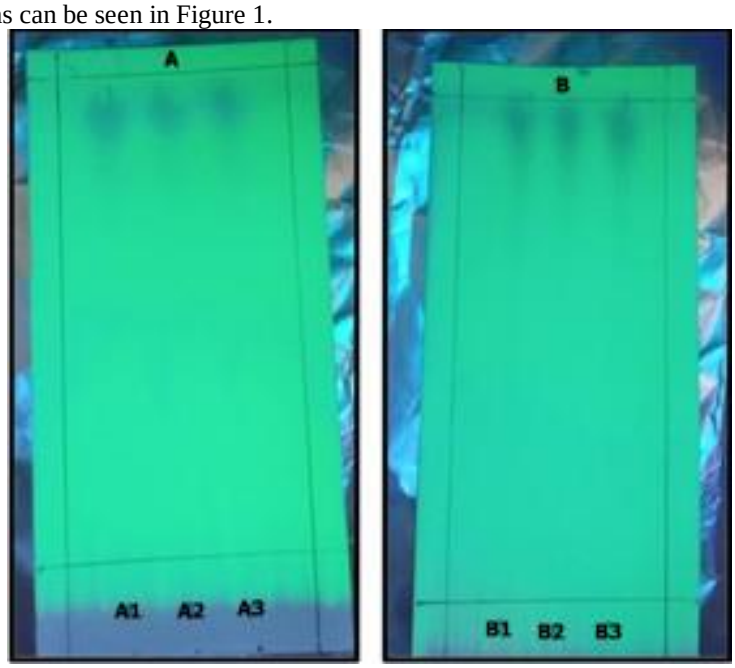

Figure 1. Observation of F. Hexane (A) and F. Ethyl acetate (B) stains

The addition of lieberman-burchad produces a bluish color under UV light 254-366 and visually yellow, The resulting reaction strengthened the identification of compounds in the n-hexane and ethyl acetate fractions that positively contained steroid saponins. Steroids will produce a bluish color after being given Lieberman-Burchad reagent and terpenoids will produce a purple color seen with UV light 254-366 (Pamungkas & Indrayudha, 2019). Wagner *et al.* (1996), stated that steroid compounds will produce a blue color, purple terpenoids, and yellow to brown phenols. The color change reaction in steroid compounds is due to the dehydration of the compound caused by the addition of a strong acid in the form of Lieberman-burchad which forms a salt and undergoes conjugation extension by giving a bluish color (Robinson, 1995). The hexane and ethyl acetate fractions produced stains with an average value of ± Rf 0.9. The Rf value was generated through the elution process of butanol: acetic acid mobile phase: aquadest (4:5:1) which is polar so that the Rf value is high in non-polar and semi-polar compounds.

According to Inayah *et al.* (2012), the spots are separated based on their degree of polarity, the polar mobile phase will produce a small Rf value if the stationary phase is polar while the spots which have a higher Rf value tend to have less polarity than the mobile phase. This confirms that based on the Rf value produced, the identified compounds are non-polar and semi-polar. The Rf value will be large if the compound is less polar and interacts with polar adsorbents from thin-layer chromatography plates. a polar mobile phase will produce a small Rf value if the stationary phase is polar, while a spot with a higher Rf value tends to be less polar than the mobile phase. This confirms that based on the Rf value produced, the identified compounds are non-polar and semi-polar. The Rf value will be large if the compound is less polar and interacts with polar adsorbents from thin-layer chromatography plates. a polar mobile phase will produce a small Rf value if the stationary phase is polar, while a spot with a higher Rf value tends to be less polar than the mobile phase. This confirms that based on the Rf value produced, the identified compounds are non-polar and semi-polar. The Rf value will be large if the compound is less polar and interacts with polar adsorbents from thin-layer chromatography plates (Handayani & Sulistyo, 2008).

4. Conclusions

The active fraction of the mattress contains steroid saponin compounds with metabolic tests on the hexane and ethyl acetate fractions showing a stable foam reaction for 10 minutes after being given 2N HCl but in the butanol fraction there are no saponin compounds characterized by no foam formation and a bluish discoloration after being given strong acid occurs in hexane and ethyl acetate fractions. The Rf value of 0.9 was obtained from TLC analysis on the hexane and ethyl acetate fractions indicating steroid saponins, the resulting single stain reacts to a bluish color after being given Lieberman-burchad (strong acid).

5. Suggestion

In this study, it is suggested to do a secondary metabolic compound test quantitatively to determine the levels of compounds in the active fraction. TLC analysis can be further improved with multi-eluent preparative TLC, the use of polar solvents is prioritized so that the stains formed are more complex.

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