ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF BIOACTIVE COMPOUND FROM SEMELE CORDIFORMIS

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Abstract: *Marine biodiversity of South East Sulawesi has been used by local communities for medication since a long time ago. One of which is Semele cordiformis or 'tude bombang' in local languages. The community believes that the hot water extract of S. cordifirmis can help to cure hepatitis. But this has not been proven scientifically. The purpose of this study is to evaluated antibacterial and antifungal activity of S. Cordiformis as a pilot project to explore the bioactivity of this biota.* ***Method****: Antibacterial activity was measured based on the Minimum Inhibitory Concentration (MIC) of S. cordiformis ethanol extract against Staphylococcus aureus ATCC 25923. Paper disks containing the extracts of S. cordiformis in several concentrations were placed on agar and the inhibition zones were measured. Antifungal activity was also measured based on the MIC of the S. cordiformis ethyl acetate extract against Candida albicans ATCC 10231. Each measurement is carried out in triplo.* ***Result****: Ethanol extract of S. cordiformis with concentrations of 50% and 100% showed strong inhibition responses (12.25 mm and 16.42 mm).Concentrations of S. cordiformis Ethyl acetate extract of 3000 mg/mL and 6000 mg/mL have moderate inhibition responses (9.58 mm and 5.42 mm).* ***Conclusion****: Semele cordiformis used in this study have potency as an antibacterial and antifungal. Further invastigation involving isolation of more specific bioactive compound of the extract need more research*

INTRODUCTION

Demanding of natural and safe food product become a trend in today's world. Products from marine-derived source is one of the most popular healthy food supplement options.[1] Marine environment provide the largest source of natural molecules to be evaluated for bioactivity in human health.[2] Marine macrobiota and microbiota are rich in bioactive

compound such as polyunsaturated fatty acids (PUFA), polysaccharides, essential vitamin and minerals, enzyme, antioxidant[3] and bioactive peptide[4].

Semele cordiformis or "tude bombang" (South East Sulawesi local language) is one of marine biota that traditionally used to cure hepatitis.[5] But this has not beed proven scientifically. The aim of this study is evaluated antibacterial and antifungal activity of *S. Cordiformis* as a pilot project to explore the bioactivity of this biota.

METHODS

Sample Collection and Preparation

S. cordiformis clamps were manually collected from local farmer in Bokori Island, Konawe, South East Sulawesi. The clamps were cracked open, the whole inner part were collected and subsequently washed with distilled water to remove attached debris. The sample was sun dried for 3-5 days and crushed to powder.[6]

Extraction of the Sample

The extraction of *S. cordiformis* powder was using maceration technique. The powder was soaked in 100% ethanol with proportion 1 part of sample and 2 part of ethanol. This maceration was conduct or 3 x 24 hrs to optimizing bioactive compound collection. A part of this maceration product was filtered using filter paper. The filtered supernatant was dried under vacuum on rotary evaporator at 50^{o}C. The result was crude ethanolic extract of *S. cordiformis.* This crude ethanolic extract will used in antibacterial activity test.

Another part of maceration product then used for fractionation. The filtered supernatant was partitioned with n-hexane (1:1) and homogenized until two layer of solution were formed. The first layer was separated from the second layer and partitioned with n-hexane. This procedure was repeated in triplo until reached a clear solution.

The remains of n-hexane fractionation was partitioned with ethyl acetate (1:1). The first layer was partitioned again in triplo with ethyl acetate until reached a clear solution. this clear solution was water fration. The n-hexane, ethyl acetate and water fraction were dried under vacuum on rotary evaporator at 50^{o}C.[7]

Identification of Bioactive Compound

To identifying the bioactive compound of the *S. semele cordiformis* extract and fraction, a Thin Layer Chromatography (TLC) was used. In this procedure, alkaloids, flavonoids, terpenoids, steroids and saponins were identified.

Antibacterial Activity Test

Etahnolic extract of *S. cordiformis* was serially diluted using distilled water in to 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% concentrations. Antibacterial activity testing was carried out using standard agar disk-diffusion assay. A *Staphylococcus aureus* ATCC 25923 suspension was prepared at concentration of 2x10^{5} CFU/ml in sterile distilled water. As much as 40 µl of bacterial suspension was inoculated on the surface of Nutrient Agar (NA). Inoculated suspension was uniformly spread on agar using a glass spreader. A sterile filter paper discs with 6 mm in diameter, impregnated with 30 µl of each ethanolic extract of *S.cordiformis* concentrations were placed on surface of inoculated plate. This discs were used to determine Minimum Inhibitory Concentration (MIC) against *S. aureus* suspension. Disc of cefadroxil and distilled water were used as positif control and negative control cosecutively. Then, plates were incubated in incubator for 24 hrs at 37ºC.

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The biaoactivity of extracts was measure by calculating the diameter (mm) of the growth inhibition zones. Zones of growth inhibition greater than 7 mm were considered susceptible to crude extracts.[8]

Antifungal Activity Test

The n-hexan, ethyl acetate, and water extract of *S. cordiformis* were serially diluted with distilled water in to 375 mg/ml, 750 mg/ml, 1500 mg/ml, 3000 mg/ml and, 6000 mg/ml concentration. Antifungal activity testing also used a standard agar disc-diffusion assays.

A spore suspension of 30 µL of *Candida albicans* ATCC 10231 was inoculated on the surface of Potato Dextrose Agar (PDA) and uniformly spread. Sterile filter paper discs with 6 mm in diameter, impregnated with 20 µl of previously diluted extract of *S. cordiformis.* Ketoconazole and distilled water containing discs were used as positif control and negative control consecutively. The plates were incubated for 72 hrs at 37ºC. Zones of growth inhibition greater than 7 mm were considered susceptible to crude extracts.[8]

There is certainly a lack of information about the molluscan secondary metabolites and its biological activities in general. The chemical constituents present in the *S. cordiformis* have not been studied so far.

RESULTS AND DISCUSSION

According to the result of bioactive compound identification, *S. cordiformis* ethanol extract contains alkaloids, flavonoids, terpenoids, and saponins. The results of the antibacterial activity test of *S. cordiformis* ethanol extract against S. aureus are shown in table 1 which shows the average diameter of the growth inhibition zone for each concentration.Antibacterial activity began to emerge at a concentration of 6.25% with an inhibition zone diameter of 2.42 mm. The diameter of the inhibition zone increases with increasing extract concentration. The MIC for S. aureus ethanol extract is 6.25% and the susceptible concentration to inhibit *S. aureus* growth is 25%. The antifungal activity test results for n-hexan, ethyl acetate, and water extract of *S. cordiformis* are shown in table 2. The n-hexan extract did not show antifungal activity as indicated by no formation of inhibition zones on PDA plates.

Table 1. *Staphylococcus aureus* growth Inhibition zone of *Semele cordiformis* ethanolic extrac

Concentration	Growth Inhibition Zone (mm)
100%	16.42
50%	12.25
25%	8.75
12.5%	5.17
6.25%	2.42
3.125%	0
1.56%	0
Cefadroxil	28.86
Distilled Water	0

Ethyl acetate extract and water extract showed an antifungal activity, but the diameter of the fungal growth inhibition zone was less than 7 mm, except for ethyl acetate extract

concentrations of 6000 mg/ml. MIC for both n-hexan and ethyl acetate extract is 375 mg / ml.

Table 2. Inhibition zone of n-hexan, ethyl acetate, and water extract of *Semele cordiformis* against *Candida albicans* ATCC 10231

Concentration	Growth Inhibition Zone (mm)		
	n-hexane	Ethyl Acetate	Water
375 mg/mL	0	1.92	2.92
750 mg/mL	0	3	3.92
1.500 mg/mL	0	4.67	5.17
3.000 mg/mL	0	5.42	5.58
6.000 mg/mL	0	9.58	5.75
Positive Control	4.08	5.75	3.75
Negative Control	0	0	0

DISCUSSION

In the present study, a pronounced antibacterial and antifungal activity has been observed against *S. aureus and C. albicans*. The ethanol extract of *S. cordiformis* has been shows activity against both bacterial and fungal strains. The MIC of *S. cordiformis* ethanol extract was observed at 6.25% concentration. Concentration of 25 % was created a mean of growth inhibition zone >7 mm. It,s mean the ethanol extract of *S. cordiformis* at concentration of approximately 25% is susceptible to inhibit the growth of *S. aureus* (Tabel 1).

In the antifungal test, ethyl acetate extract and water extract proved to be more effective in inhibiting the growth of *C. albicans* compared to n-hexan extract (Table 2). The n-hexane is non-polar solvent so it tends to only extract non-polar compound such as steroids and terpenoids. While the ethyl acetate solvent is semipolar and suitable to extract polar, semipolar, and nonpolar compounds such as semipolar alkaloids. The last fraction is the water solvent which has only extract polar compounds such as saponins and flavonoids. According to TLC analysis, the etahonol extract of *S. cordiformis* contain bioactive compound such as alkaloids, flavonoids, terpenoids, and saponins.

Alkaloid are diverse group of amino acid-derived and nitrogen-bearing molecules that display a wide range roles in nature.[9] Alkaloid naturally founds in plants, animals, marine organism, and microorganism.[10] Alkaloids are basics and possess a nitrogen atom with an unshared pair of electrons.[11,12] Alkaloids are insoluble or sparingly soluble in water, unless reacted with an acid to form a salt. Alkaloids are soluble in non-polar solvents such as chloroform, but their salts are not.[12]

Antibacterial activity of alkaloids is obtained from the susceptibility to form hydrogen bond with proteins, enzymes and receptors because possessing a proton-accepting nitrogen atom and one or more proton-donating amine hydrogen atoms. This, coupled with the frequent presence of proton-accepting and-donating functional groups such as phenolic hydroxyl and polycyclic moieties.[13]

Flavonoids are low molecular weight compounds, consisting of fifteen carbon atoms, arranged in a C6–C3–C6 configuration.[14] Most of them have several bioactivity such as anti-oxidant, antiinflammation, anticancer and cardiovascular protection.[15] Flavonoids are important antioxidants due to their high redox potential, which allows them to act as

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reducing agents, hydrogen donors, and singlet oxygen quenchers.[16] Flavonoids can be further classified into anthocyanins, flavones, isoflavones, flavanones, flavonols and flavanols.[17] Flavonoids also have antibacterial bioactivity that can be exerted by directly kill the bacteria, synergistically activate the antibiotics, and attenuate the bacterial pathogenicity.[18]

Flavonoids are the most commonly found phytochemicals, that help to protect the plant against UV light, fungal parasites, herbivores, pathogens and oxidative cell injury.[19] Despite commonly found in plants, an investigations indicate the presence of flavonoids in marine flora. Several flavonol glycosides, including quercetin derivatives, have been identified in the fresh water microalga Hematococcus pluvialis.[20] Flavonol glycosides are compounds where a sugar molecule is bound with the flavonoid group through a glycosidic bond. In addition, isoflavones have also been identified from several marine and fresh water algae and cyanobacteria.[21,22]

Saponins are freely soluble in both organic solvents and water. it's have antifungal[23], antibacterial[24], anti-inflammatory activity[25] and also anticarsinogenic[26]. This compound may cause membrane perturbation by the formation of pores on the membrane.[27] Based on their observation onformation of pores or pits on the membrane, some study concurrently reported that the presence of cholesterol on the target membrane is essential for the saponins to induce pore formation.[28,29] Saponin are mainly produced by plants, but also by lower marine animals and some bacteria[30,31].

Terpenes are naturally occurring substances produced by a wide variety of plants and animals. A broad range of the biological properties of terpenoids is described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic activities. The extracts were also positive for steroids, which are very important compounds especially due to their relationship with compounds such as sex hormone.[32]

Bivalve species consume nutrients from many material particles, such as phytoplankton, resuspended benthic microalgae, and detritus from both bacterial and myco-heterotrophic sources, and phytoplankton is their primary food source.[33] The food source maybe have a responsibility that is flavonoid found in bivalve species.

CONCLUSION

Semele cordiformis used in this study have potency as an antibacterial and antifungal. Further invastigation involving isolation of more specific bioactive compound of the extract need more research.

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