



Antifungal Activity Test of Ethanol Extracts and Ethyl Acetate and N-Hexane Fraction of Sea Grapes (*Caulerpa Racemosa*) on the Growth of the Fungi *Trichophyton mentagrophytes*

Sri Hainil¹, Delladari Mayefis¹, Habibie Deswilyaz Ghiffari¹, Roza Erda², Tessa Leondri^{1*} , Anjeli Firanika¹

¹Bachelor of Pharmacy Study Program, Mitra Bunda Health Institute, Batam, Riau Islands, Indonesia; ²Bachelor of Nursing Study Program, Mitra Bunda Health Institute, Batam, Riau Islands, Indonesia

Abstract

Edited by: Slavica Hristomanova-Mitkovska
Citation: Hainil S, Mayefis D, Ghiffari HD, Leondri T, Firanika A. AntiFungal Activity Test of Ethanol Extracts and Ethyl Acetate and N-Hexane Fraction of Sea Grapes (*Caulerpa Racemosa*) on the Growth of the Fungi *Trichophyton Mentagrophytes*. Open Access Maced J Med Sci. 2023 Jan 02; 11(A):76-82. https://doi.org/10.3889/oamjms.2023.11250

Keywords: Antifungal; Sea grape; *Trichophyton mentagrophytes*

***Correspondence:** Tessa Leondri, Bachelor of Pharmacy Study Program 2018-2022, Mitra Bunda Health Institute, Batam, Riau Islands, Indonesia.
E-mail: tleondri@gmail.com

Received: 12-Nov-2022

Revised: 28-Nov-2022

Accepted: 21-Dec-2022

Copyright: © 2023 Sri Hainil, Delladari Mayefis, Habibie Deswilyaz Ghiffari, Tessa Leondri, Anjeli Firanika
Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

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BACKGROUND: Sea grape (*Caulerpa racemosa*) is one of the marine plants that produce active compounds that can be used as medicinal ingredients with antifungal properties to inhibit fungal growth.

AIM: The purpose of this study was to determine whether the ethanolic extract of the ethyl acetate fraction and n-Hexane of sea grapes taken from Kampung Terih, Riau Islands could inhibit the growth of the fungus *Trichophyton mentagrophytes*.

METHODS: The antifungal activity was tested using the paper disk diffusion method with variant concentrations of ethanol extract, namely, 25%, 50%, 75%, and 100%, ethyl acetate and n-hexane fractions were 20,000 ppm, 30,000 ppm, 40,000 ppm, and 50,000 ppm.

RESULTS: The results showed that the ethanolic extract of sea grapes could inhibit the growth of *T. mentagrophytes* fungus with the formation of an average diameter at each concentration of 0 mm; 3.4 mm; 5.4 mm; and 8.4 mm. The ethyl acetate fraction of Sea grapes formed an average diameter at each concentration of 9.7 mm; 11.51 mm; 13.5 mm; 15.8 mm.

CONCLUSION: Based on the results of the study, it was concluded that the ethanol extract and the ethyl acetate fraction of sea grapes could inhibit the fungus *T. mentagrophytes* with moderate inhibition, while the n-hexane fraction of sea grapes could not inhibit the fungus *T. mentagrophytes*.

Introduction

In Indonesia, the Riau Archipelago is one of the provinces that have abundant natural resources, especially marine products. One of the marine products owned is sea grapes. This plant grows in clusters so it is called sea grapes [26]. The sea grapes in Terih Nongsa Village are called Latoh or Lawi lawi, these sea grapes are used as fresh vegetables which are believed to prevent premature aging.

The existence of sea grapes, especially in the waters of the Riau Islands, is very abundant, especially on beaches that have flat coral reefs. Phytochemical analysis of *Caulerpa* sp, showed that this plant contains secondary metabolites [13]. Sea grapes (*Caulerpa racemosa*) contain active compounds such as saponins and flavonoids that have potential as antifungals.

Infections that often occur in the community are usually caused by fungi or fungi [19]. One of the diseases caused by fungi is dermatophytosis, a skin disease caused by the dermatophyte group of fungi.

This group of fungi can live in beach sand, so they are often found in coastal areas [22]. *Trichophyton mentagrophytes* is a type of dermatophyte that often infects humans [25].

The results of previous research conducted by Majula and Rao [11] stated that the *Caulerpa taxifolia* algae extract provided antibacterial activity against Gram-positive and negative bacteria as well as antifungals using chloroform, methanol, n-hexane, and water solvents against the fungus *Candida albicans* with an inhibition zone diameter of 21 mm and *C. racemosa* algae extract provided antibacterial activity against Gram-positive and negative bacteria, and antifungal against the fungus *Aspergillus niger* with an inhibition zone diameter of 22 mm. According to research from Siagian [20] stated that the ethanol extract of sea grapes (*C. racemosa*) from Ambai Serui Island had antifungal activity against *C. albicans* and *Candida krusei* at a concentration of 1000 ppm with an inhibition zone of 8.55 mm. and 9.44 mm.

Based on the previous research, no research has been found to test the antifungal activity of extracts of sea grapes (*C. racemosa*) using the fungus *T.*

mentagrophytes, especially in the area of Kampung Terih, Nongsa Beach, Batam, Riau Islands. So that, researchers are interested in conducting research on the antifungal activity of ethanol extract of sea grapes (*C. racemosa*) against the growth of the fungus *T. mentagrophytes* using the disk paper diffusion method with concentrations of 25%, 50%, 75%, and 100% for ethanol extract and concentrations of 20,000 ppm, 30,000 ppm, 40,000 ppm, and 50,000 ppm for the ethyl acetate and n-hexane fractions. So this research was conducted to find out at what concentration the ethanol extract of the thyl acetate fraction and n-hexane of sea grapes (*C. racemosa*) had the largest inhibition zone in inhibiting the growth of the fungus *T. mentagrophytes*.

Materials and Methods

Tools

The tools used are autoclave, Erlenmeyer, hot plate (Maspion s.302), Petri dish, rotary evaporator (Heidolph made in Germany), digital scale (Kenko), vortex mixer, test tube, test tube rack, oven, sterile cotton, ose needle, laminar air flow (LAF) (Magnehelic), Bunsen, aluminum foil, magnetic stirrer, incubator (Memmert), caliper, porcelain crucible, paper disk, stir bar, filter cloth, and glass jar.

Ingredients

The ingredients used are sea grape (*C. racemosa*), 95% ethanol, ethyl acetate, n-hexane, H₂SO₄ (Sulfuric Acid), ammonia, Mg (Magnesium) powder, Meyer reagent, HCl (Chloric Acid), aquadest, chloroform, chloral hydrate, FeCl₃ (Iron III Chloride), media Sabouraud dextrose agar (SDA), BaCl₂ (Barium Chloride) 1%, CH₃COOH (Acetic Acid), dimethyl sulfoxide (DMSO) 10%, ketoconazole 2%, *T. mentagrophytes* fungi, and NaCl (Sodium Chloride) 0.9%.

Sample preparation

Samples of sea grapes (*Caulerpa rasemosa*) that have been collected are sorted and washed under running water to free from mud, then sea grapes are drained and weighed; initially, then, the sea grapes are chopped into small pieces (Hainil et al., 2021).

Sea grapes extract making

Sea grapes (*C. racemosa*) were weighed as much as 20 kg then cut into small pieces, then put into a glass container and macerated using

ethanol solvent until all parts of the sea grape were submerged. Maceration was carried out for 3 days, stirring every day. Every 3 days the filtrate was filtered and the dregs were macerated again with ethanol. The maceration process was repeated 3 times. The results of the macerate obtained from the three macerations were combined and the solvent was evaporated using a rotary evaporator to obtain a thick extract of sea grapes. After the thick extract was found, the extract was then weighed to get the percentage yield.

Manufacturing of ethyl acetate and n-hexane fraction

The thick ethanol extract obtained was taken as much as 10 g, added 100 ml of distilled water, stirred until homogeneous, then put into a separating funnel and fractionated with 100 ml of n-hexane solvent and shaken slowly and then allowed to stand until two layers were formed consisting of the n-hexane fraction and water fraction. The layers of n-hexane fraction and water fraction were separated. Do the addition of n-hexane repeatedly until a clear n-hexane fraction is obtained. Then, the remaining water layer of the n-hexane fraction was fractionated again with 100 ml of ethyl acetate as a solvent. Ethyl acetate was added repeatedly until a clear ethyl acetate fraction was obtained. Then, the water layer is evaporated over a water bath until it becomes thick, which is then called the water fraction [7].

Extract characterization

Organoleptic examination

Physical identification was carried out with the five senses including smell, shape, and color of the extract [6].

Moisture check

A sample of 2 g was weighed and placed in a porcelain dish. Then dried in the oven at a temperature of 100–105°C for 3 h. Then cooled in a desiccator for 15 min and then weighed. This treatment was repeated until a constant weight was reached [6].

Determination of total ash content

Weigh the thick extract as much as 2 g and then put it in a porcelain crucible which had previously been heated at 105°C for 30 min and has been thawed. After that, the porcelain crucible was put into the furnace and then ignited at 600°C for 7 h, removed, and cooled in a desiccator for 15 min and weighed again [6].

Phytochemical screening

Alkaloid test

Take 40 mg of sea grape extract (*C. racemosa*) put into a test tube, add 2 mL of ammonia, and 2 mL of chloroform. Then, 3–5 drops of concentrated H_2SO_4 (Sulfuric acid) then shaken and leave for a while to form two layers. The top layer was transferred to a test tube and 4–5 drops of Mayer's reagent were added. If there is a white precipitate, it indicates a positive alkaloid [6].

Flavonoid test

Take 40 mg of sea grape extract (*C. racemosa*) put into 20 mL of distilled water, heated for 5 min in a test tube. Then added 1 mL of concentrated HCL (Hydrochloric acid) and 0.2 g of Magnesium powder. If there is a color change to dark red (magenta) within 3 min, it indicates a positive flavonoid [6].

Fenolik test

Take 40 mg of sea grape extract (*C. racemosa*) and put it in a test tube. Add 10 drops of 1% $FeCl_3$. If there is a color change to green, red, purple, blue, or solid black, it indicates a positive phenol [6].

Saponin test

Take 40 mg of sea grape extract (*C. racemosa*) and put it in a test tube. Then add 10 mL of hot water, cool, then shake vigorously for 10 s. If positive for saponins, it will be indicated by the formation of foam that lasts no <10 min [6].

Terpenoid and steroid test

Take 40 mg of sea grape extract (*C. racemosa*) and add 10 drops of CH_3COOH (acetic acid) and 2–3 drops of H_2SO_4 (sulfuric acid). Then shaken gently and leave for a few minutes. If a red or purple color is formed, it indicates a positive terpenoid and if a blue color is formed, it indicates a steroid positive [6].

Sterilization

The tools to be sterilized are washed thoroughly first to remove impurities, then dried until they are completely dry. Then wrap the tool using aluminum foil or paper. Then put the tool that has been wrapped in aluminum foil or paper into an autoclave to be sterilized at 121°C for 15 min. Tools made of rubber are sterilized by immersing them in 70% alcohol. Ose needles are sterilized by incandescent using a Bunsen flame. LAF was sterilized by UV lamp for 15 min and sprayed with 70% alcohol. Laminar sterilization is carried out before and after working in it.

Making of SDA media

The preparation of agar media was carried out by means of SDA media dissolved with aquadest into an erlenmeyer. Then the mixture is heated on a hot plate so that it is homogeneous until it boils for ± 40 min. The agar medium was sterilized in an autoclave at 121°C for 20 min. After sterilization, the media was then cooled until the temperature reached 45°C, then 20 mL each was poured into a Petri dish and 15 mL into a test tube as a media for tilting. SDA media that has been poured into Petri dishes and test tubes is allowed to harden [19].

Fungal rejuvenation

Before an antifungal test is carried out, the fungus to be used must be regenerated first. The first thing that must be done is to make SDA media slanted, namely, by streaking one ose culture from pure culture of *T. mentagrophytes* on fresh slanted SDA media and then incubated at 25–27°C for 3–5 days in the incubator [12].

Standard building Mc. Farland

Enter 0.05 mL of 1% $BaCl_2$ (Barium chloride) solution and mix it with 9.95 mL of 1% H_2SO_4 (Sulfuric acid) solution into a test tube, then homogenize until a cloudy solution is formed. This solution was used as a standard for the turbidity of the fungal test [18].

Making fungi suspension

Fungal cultures that have been aged for 3–5 days are taken from tilted agar as much as two oses of test fungus colonies suspended in 5 mL of sterile 0.9% NaCl in a test tube. Then homogenized with a vortex. Turbidity compared to Mc. Farland [16].

Antifungal activity test

Testing for antifungal potential was carried out by the method (disk diffusion) using disc paper. Using a positive control of 2% Ketoconazole and a negative control of DMSO 10%. The first procedure was a sterile Petri dish, the edges were heated using a Bunsen flame. Then as much as 20 mL of SDA was put into each Petri dish. The fungal suspension that had been made previously was taken as much as 50 μ L and then dripped on the center of the agar surface, then spread using sterile cotton so that the distribution of the fungal test was more evenly distributed [2]. After that, sterile disk paper was placed that had been dripped with 20 μ L sea grape extract with concentrations for 25%, 50%, 75%, and 100% ethanol extract, while the concentrations for the fractions were 20,000 ppm, 30,000 ppm, 40,000 ppm, and 50,000 ppm [10]. As a

negative control, DMSO 10% was used and a positive control was used ketoconazole 2%. Repeat 3 times. Then, the Petri dishes were incubated in an incubator for 72 h at a temperature of 25–27°C. Then, the antifungal activity was determined by measuring the diameter of the inhibition zone formed using a caliper [21].

Results

Extract characterization

The characteristics of the extracts carried out in this study were in the form of organoleptic tests, ash content tests, and water content tests. The purpose of carrying out the characteristics of the extract is to determine the quality of a simplicia and extract [13]. The results of the organoleptic test showed the form of a thick extract of Sea Grape (*C. racemosa*) obtained thick and contained crystals, green-brown in color, and fishy smell and salty taste in Figure 1.



Figure 1: Organoleptic examination

The results of the ash content test obtained were 16.91% in Table 1, and the results of the water content test obtained are 19.87% in Table 2.

Table 1: Results of extract ash content

Empty crucible weight (A)	Crust weight+extract before oven (B)	Crust weight+extract after oven (C)	% ash content
53.885 g	55.215 g	1=54.270 g 2=54.110 g 3=54.110 g	16.91

Phytochemical screening

Phytochemical screening test is carried out to determine the class of substances or compounds

Table 2: Results of extract moisture content

Empty crucible weight (A)	Crust weight+extract before oven (B)	Crust weight+extract after oven (C)	% water content
30.205 g	32.645 g	1=32.540 g 2=32.160 g 3=32.160 g	19.87

contained in the sample to be studied. Phytochemical screening test is carried out by color testing using a color reagent [9]. The results of phytochemical screening of ethyl acetate and n-hexane fractions of sea grape ethanol extract showed positive results on metabolites secondary alkaloids, flavonoids, saponins, and phenolics and showed negative results on steroids and terpenoids (Tables 3-5).

Antifungal activity test

Antifungal activity testing aimed at determining the ability of the ethyl acetate and n-hexane fractions of sea grape ethanol extract (*C. racemosa*) to inhibit the fungus to be tested. The ability of the extract to inhibit fungi was seen from the clear zone around the paper disk. The antifungal activity of the ethanolic extract of sea grape (*C. racemosa*) was tested against the fungus *T. mentagrophytes*. This research was carried out 3 times with four concentrations for the ethanol extract, namely, 25%, 50%, 75%, and 100%, and four concentrations for the ethyl acetate and n-hexane fractions, namely, 20,000 ppm, 30,000 ppm, 40,000 ppm, and 50,000 ppm, Ketoconazole 2% positive control and 10% DMSO negative control (Figures 2-4).

In this study, the results of the measurement of the inhibition zone of the ethanolic extract of sea grapes (*C. racemosa*) on the fungus *T. mentagrophytes* at concentration 25% no inhibition zone was obtained, at 50% concentration, an inhibition zone was obtained of 3.4 mm (the antifungal category is classified as

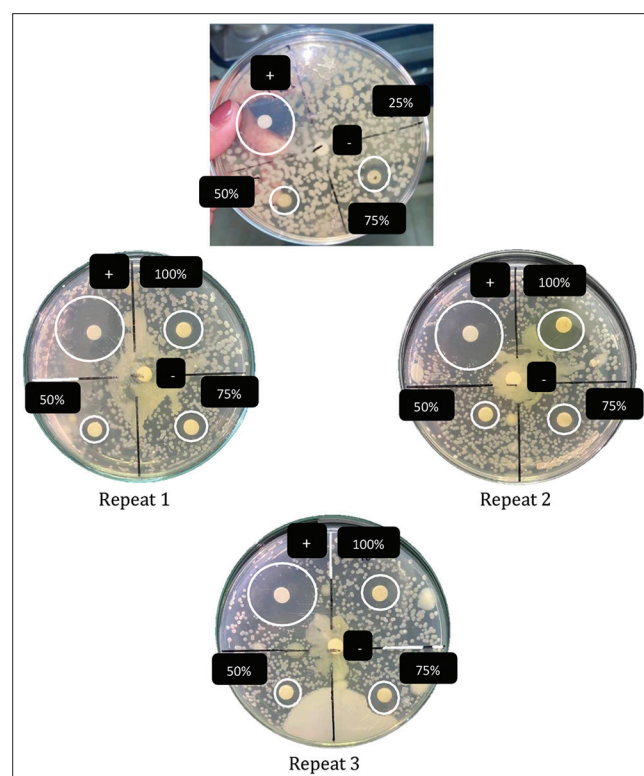


Figure 2: Test results of sea grape ethanol extract on the fungus *trichophyton mentagrophytes*

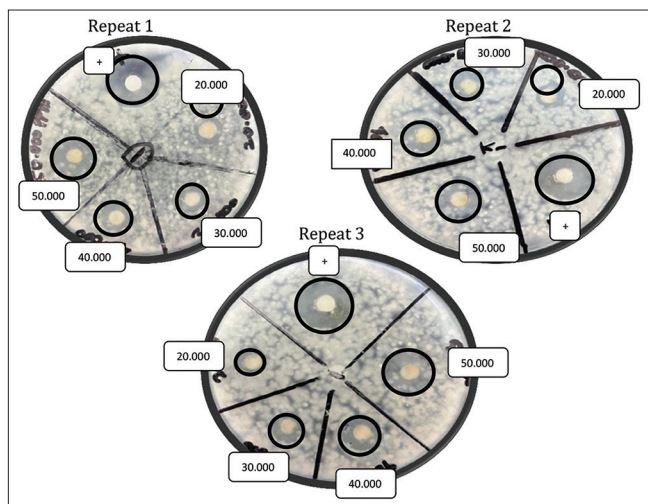


Figure 3: Test results of sea grape ethyl acetate fraction against trichophyton mentagrophytes

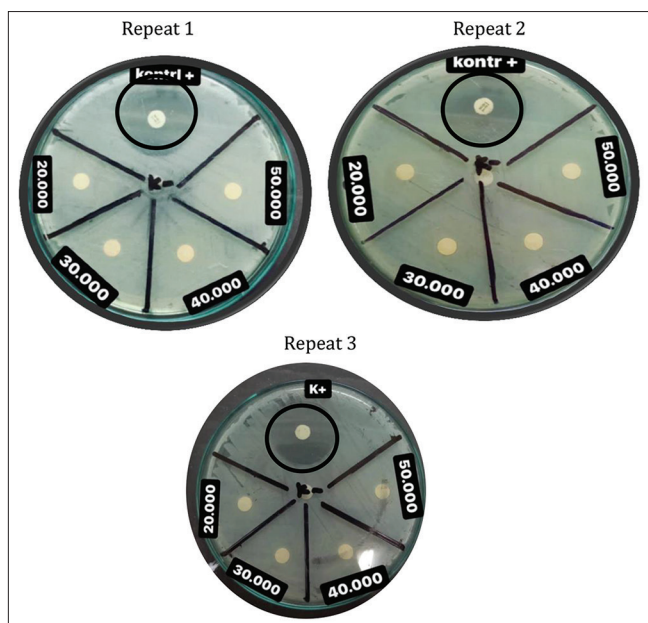


Figure 4: Test results of sea grape n-hexane fraction against trichophyton mentagrophytes

weak), at a concentration of 75%, an inhibition zone of 5.4 mm is obtained (the antifungal category is classified as moderate) and has the largest inhibition zone at 100% extract concentration, which is 8.4 mm (medium antifungal category) (Table 6).

Table 3: Results of phytochemical screening of sea grape ethanol extract

Secondary metabolites	Testing method	Results
Alkaloid	Ammonia+chloroform+sulfuric acid+Mayer's reagent	Positive
Flavonoid	Concentrated HCL (Hydrochloric acid) + Mg (magnesium) Powder	Positive
Saponin	Hot aquadest	Positive
Steroid	Acetic acid+H ₂ SO ₄ (sulfuric acid)	Negative
Terpenoid	Acetic acid+H ₂ SO ₄ (sulfuric acid)	Negative
Fenolik	FeCl ₃ 1%	Positive

The results of the measurement of the inhibition zone on the ethyl acetate fraction having the largest inhibition zone at a concentration of 50,000 ppm obtained an

inhibitory diameter of 15.8 mm (Table 7). The concentration is more optimum, because at that concentration, the level of antifungal compounds that have the potential as antifungals is sufficient to inhibit the growth of fungi.

While the repetition of various concentrations showed no antifungal activity of the n-hexane fraction at concentrations of 20,000 ppm, 30,000 ppm, 40,000 ppm, and 50,000 ppm (Table 8). Hence, this study showed that the n-hexane fraction of sea grapes (*C. racemosa*) could not inhibit the growth of the fungus *T. mentagrophytes*.

Table 4: Results of phytochemical screening of sea grape ethyl acetate fraction

Secondary metabolites	Testing method	Results
Alkaloid	Ammonia+chloroform+sulfuric acid+Mayer's reagent	Positive
Flavonoid	Concentrated HCL (Hydrochloric acid) + Mg (Magnesium) Powder	Positive
Saponin	Hot aquadest	Positive
Steroid	Acetic acid+H ₂ SO ₄ (Sulfuric acid)	Negative
Terpenoid	Acetic acid+H ₂ SO ₄ (Sulfuric acid)	Negative
Fenolik	FeCl ₃ 1%	Positive

Discussion

This study was conducted to determine the antifungal activity of ethanol extract of ethyl acetate and n-hexane fractions Sea grape (*C. racemosa*) in inhibiting fungi *T. mentagrophytes*. The extraction method used in this research is the method maceration. The reason for choosing the maceration method in the extraction process is because the tools used are simple and do not go through a heating process so as to minimize damage. The characteristics of the extracts carried out in this study were in the form of test organoleptic test, ash content test, and water content test. The purpose of doing characteristics extract is to determine the quality of a simplicia and extract [13].

Table 5: Results of phytochemical screening of sea grape n-hexane fraction

Secondary metabolites	Testing method	Results
Alkaloid	Ammonia+chloroform+sulfuric acid+Mayer's reagent	Positive
Flavonoid	Concentrated HCL (hydrochloric acid) + Mg (magnesium) powder	Positive
Saponin	Hot Aquadest	Positive
Steroid	Acetic acid+H ₂ SO ₄ (sulfuric acid)	Negative
Terpenoid	Acetic acid+H ₂ SO ₄ (sulfuric acid)	Negative
Fenolik	FeCl ₃ 1%	Positive

The results of the ash content test obtained are 16.91%. In line with the statement of Venugopal [24], the majority of good mineral nutritional value in seaweed types is the ash content with a value between 8.4 and 43.6%. Moreover, the results of the water content test obtained are 19.87%, in line with the opinion of Voight [25] which states that the value of water content which is good for liquid extracts of more than 30%, viscous extracts ranging from 5 to 30%, and dry extract <5%.

The results of phytochemical screening showed positive results on metabolite compounds secondary

Table 6: Results of measurement of inhibitory power of sea grape ethanol extract

Treatment	Average inhibition zone diameter (mm)	Growth barrier response
Concentration 25%	-	-
Concentration 50%	3.4 mm	Weak
Concentration 75%	5.4 mm	Currently
Concentration 100%	8.4 mm	Currently
Control (+) (ketokonazole)	20.6 mm	Very strong
Control (-) (DMSO 10%)	-	-

alkaloids, flavonoids, saponins, and phenolics and showed negative results on steroids and terpenoids. Mechanism of action of alkaloids as antifungals which interferes with fungal growth by preventing fungal DNA replication, so that cell division does not occur and the fungus fails to grow [17]. According to Siagian [20], flavonoids and saponins have potential as antimicrobials, flavonoids interact with microbial cells to form phenol-protein complexes and penetration of phenol into cells causes protein coagulation so that the cell membrane undergoes lysis. The mechanism of saponins as antifungals occurs through interference with cell membrane so that the cell undergoes lysis or is damaged and the cell membrane ruptures so that important components that exist in microbial cells such as proteins and acids nucleic acid released, resulting in microbial death.

Table 7: Results of measurement of inhibitory power of sea grape ethyl acetate fraction

Treatment	Average inhibition zone diameter (mm)	Growth barrier response
Concentration 20,000 ppm	9.7 mm	Weak
Concentration 30,000 ppm	11.51 mm	Weak
Concentration 40,000 ppm	13.5 mm	Weak
Concentration 50,000 ppm	15.8 mm	Currently
Control (+) (ketokonazol)	17.8 mm	Currently
Control (-) (DMSO 10%)	-	-

The positive control used in this study was ketoconazole 2% and the negative control is DMSO 10%. The reason for choosing ketoconazole as positive control because ketoconazole is an azole antifungal drug. It has a broad-spectrum against several fungal species and is widely used as the treatment of superficial and systemic infections.

While the reason for choosing DMSO as a negative control was because according to Octaviani [15] DMSO did not provide activity against fungal growth and bacteria so that it will not interfere with the results of research observations.

In this study as a whole with repetition of various variations, the concentration showed antifungal activity in the ethanol extract and ethyl acetate fraction with the formation of an inhibition zone, while the n-hexane fraction did not form an inhibition zone.

Table 8: Results of measurement of inhibitory power of sea grape n-hexane fraction

Treatment	Average inhibition zone diameter (mm)	Growth barrier response
Concentration 20,000 ppm	-	-
Concentration 30,000 ppm	-	-
Concentration 40,000 ppm	-	-
Concentration 50,000 ppm	-	-
Control (+) (ketokonazol)	17.8 mm	Currently
Control (-) (DMSO 10%)	-	-

Hence, this study shows that the ethanol extract of the ethyl acetate fraction of sea grapes (*C. racemosa*) can inhibit the fungus *T. mentagrophytes* using the disk diffusion method.

Acknowledgments

Thank you and grateful for the support of all the Mitra Bunda Institute of Health who has provided research facilities.

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