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ANTIBACTERIAL ACTIVITY EXTRACT SEA GRAPE (*Caulerpa racemosa*) AGAINTS *Micrococcus luteus* AND *Klebsiella pneumoniae*

Sri Hainil [1)], Mawardi Badar[2)], Hesti Marliza[3*)], Rastria Meilanda[4)], Apriyani Efendi[5)]

Institut Kesehatan Mitra Bunda, Jl.Raya Seraya No 1, Tlk Tering, Kec Batam Kota, Kota Batam, Kepulauan Riau 2944.

*Email: **hesti79id@gmail.com**

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Penulis Korespondensi

Name : Sri Hainil
Affiliation : Universitas Perintis Indonesia
E-mail : **nininkda72@gmail.com**

A B S T R A C T

Research on the antibacterial activity of Sea Grapes (Caulerpa racemosa) against Klebsiella pneumoniae and Micrococcus luteus is crucial due to the significant potential hazards posed by these bacteria. Klebsiella pneumoniae, a Gram-negative bacterium, can cause serious infections in the urinary and respiratory tracts, while Micrococcus luteus, a Gram-positive bacterium, often infects the skin of fish and humans. Sea Grapes, found in various Indonesian waters, contain secondary metabolites with potential as bioactive compounds in pharmaceuticals, including antibacterial, antiviral, and antifungal agents. The high alkaloid content, especially Caulerpin, in C. racemosa demonstrates substantial potential for developing new antibacterial agents. Although previous research has shown moderate antibacterial activity against Staphylococcus aureus and ineffectiveness against Escherichia coli, further exploration of other bacteria such as Klebsiella pneumoniae and Micrococcus luteus could open new opportunities for discovering effective antibacterial compounds. The purpose of this research to know antibacterial activity fractions of sea grapes. The bacteria used were Micrococcus luteus and Klebsiella pneumoniae. The method used is paper disc diffusion method. The antibacterial activity was tested using extract with concentration 25%, 75%, 100% and used a positive control Tetracycline 30 g/paper disk and a negative control using DMSO 10%. The results of the extract tested were the most active as an antibacterial extract with a concentration of 100% on Micrococcus luteus bacteria with an inhibitory power of 16.7 mm and on Klebsiella pneumoniae bacteria which had an inhibitory power of 12.1% mm. The conclusion of this reseach the extract had antibacterial activity

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INTRODUCTION

Indonesia is a country that has a lot of abundant marine wealth, one of which is from Kampung Terih Nongsa, Batam, Riau Islands. Biodiversity owned is Sea Grape (*Caulerpa racemosa*). In the people of Kampung Terih Laut Grape it is used as fresh vegetables, anti-aging, and some use it as an itching medicine.

Sea Grape (*Caulerpa racemosa)* is a type of green algae that lives in several Indonesian waters. Alga *Caulerpa racemosa* has characteristics such as a green thallus like a grass plant, has many upright branches and at the top of the branch there are rounded like grapes (Sirait et al., 2022)

Sea grapes contain secondary metabolites that are used as a source of bioactive compounds in the pharmaceutical field such as antibacterial, antiviral and antifungal (Zainuddin dan Malina, A, 2009). Alkaloids were the dominant antibacterial in *Caulerpa racemosa* at 7.78 - 8.94 mg/g. The high content of alkaloids in *Caulerpa racemosa* is due to the presence of Caulerpin which is quite high in this type of algae (Balansa, 2023).

Research conducted by (Ritan et al., 2021) found that *Caulerpa racemosa* had moderate antibacterial activity against *Staphylococcus aureus,* but did not have antibacterial activity against *Escherichia coli* bacteria. According to (Izzati, 2007) the results of research on antibacterial activity tests on marine algae *Caulerpa racemosa*, have compounds containing antibacterial activity *on Edwardsiela tarda, Yersinia enterocolitica and Proteus stuartii*.(Hainil et al., 2023)

Antibacterial is a compound used to control the growth of harmful bacteria (Karunakaran et al., 2022). *Klebsiella pneumoniae* is a Gram-negative bacterium that can cause urinary tract and respiratory infections . *Micrococcus luteus* is a Gram-positive bacterium that is often found infecting fish skin and human skin (Liu et al., 2023).

The purpose of this research was to determine the effectiveness of the Sea Grape fraction in Terih Nongsa Village in inhibiting the growth of *Micrococcus luteus* and *Klebsiella pneumoniae* bacteria.

METHODS

Equipment

Autoclave, Erlenmeyer, hot plate (*Maspion s.302*), petri dish, Rotary evaporator (*Heidolph made in Germany*), digital scale (*Kenko*), vortex mixer, filter, test tube, test tube rack, oven, needle loop, LAF (Laminar Air Flow) (*magnehelic*), Bunsen, aluminum foil, magnetic stirrer, filter paper, incubator (*memmert*), caliper, microscope (*Olympus*), porcelain exchange, disc paper, slide, glass cover, stirring rod, glass container, tweezers, cotton sterile, micro pipette (*adjust)*, separating funnel.

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Ingredient

Sea Grape (*Caulerpa racemosa*), 95% ethanol, Ethyl acetate, n-Hexane, Nutrient Agar (NA) (*HiMedia*), H2SO4 (sulfuric acid), ammonia, Mg (Magnesium) powder, Mayer's reagent, concentrated HCl (hydrochloric acid) , aquadest, chloroform, FeCl3 (Iron III Chloride), BaCl2 1% (Barium Chloride), CH_3COOH (acetic acid), NaCl (Sodium chloride) 0.9%, sterile distilled water, Tetracycline paper disk, bacteria *Klebsiella pneumoniae* and *Micrococcus luteus.*

Sampling

The sample used in this study was Sea Grape (*Caulerpa racemosa*) located in the area of Kampung Terih, Nongsa Beach, Riau Islands. All parts of the Sea Grape plant were taken as much as 20 kg.

Sample setup

The collected Sea Grape (*Caulerpa racemosa*) samples were sorted and washed under running water to free from mud, then the Sea Grape was drained and weighed initially, then the Sea Grape was chopped into small pieces.

Extraction

Sea Grape (*Caulerpa racemosa*) as much as 20 kg was cut into small pieces, then put into a glass container and macerated using 95% ethanol solvent until all parts of the Sea Grape were submerged. Maceration was carried out for 3 days, stirring every day. Every 3 days it was filtered and the dregs were macerated again with 95% 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 Grape. The yield obtained is calculated based on the percentage of weight (w/w) using the following equation:

$$\textbf{\% Yield} = \frac{\text{Weight of thick extract obtained}}{\text{weight of sample used}} \times \textbf{100\%}$$

Phytochemical Screening

Phytochemical Screening Test (Sri Hainil, 2021):

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1. Alkaloid Test

Sea Grape thick extract as much as 20 mg plus 2 ml of ammonia and 2 ml of chloroform into a test tube, add 3-5 drops of concentrated sulfuric acid then shake and leave for a while to form 2 layers. The top layer was transferred to a test tube and 4-5 drops of Mayer reagent were added, then if a white precipitate was formed, it indicated the presence of alkaloids.

2. Flavanoid Test

The thick extract of Sea Grape 20 mg was added to 20 ml of distilled water and then heated for 5 minutes in a test tube. Then 1 ml of concentrated HCL was added and 0.2 g of Mg powder was added. If within 3 minutes a dark red (magenta) color appears, it indicates that the ethanolic extract of sea grapes contains flavonoids.

3. Phenolic Test

As much as 20 mg of Sea Grape extract was added 10 drops of 1% FeCl3. Positive extracts contain phenol if they produce green, red, purple, blue or solid black colors.

4. Saponin Test

Sea Grape thick extract as much as 20 mg is put into a test tube, add 10 ml of hot water, then shake vigorously for 10 seconds. Add 1 drop of 2N HCl. The presence of saponins is indicated by the formation of foam that lasts not less than 10 minutes.

5. Terpenoid Test

Sea Grape thick extract 20 mg added 10 drops of acetic acid and added 2-3 drops of sulfuric acid. Then shake gently and leave for a few minutes. If a red or purple color is formed, it contains terpenoids.

Tool Sterilization

The tools to be used are washed and then dried and sterilized first. Cover all tools with aluminum foil and sterilize using autoclave at 121ºC for 15 minutes or oven at 170ºC for 1 hour.

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Making Nutrient Agar Media

The NA used was Nutrient Agar 28gr/1000 ml. The preparation of agar media was carried out by means of 6.72 grams of nutrient agar dissolved in 240 ml of aquadest into an erlenmeyer. Then the mixture is heated on a hot plate so that it is homogeneous until it boils for ± 40 minutes. The agar medium was sterilized in an autoclave at 121ºC for 15 minutes. After sterilization, the media was then cooled until the temperature reached 45^{o} C, then 20 ml each was poured into 12 petri dishes. Nutrient Agar (NA) media that has been poured into a petri dish is allowed to harden (Hainil et al., 2021).

Bacterial Rejuvenation

Before the antibacterial test was carried out, take the bacteria Micrococcus luteus and Klebsiella pneumoniae from pure culture, each test bacteria was taken as much as one ose and then inoculated by scratching on the NA (Nutrient Agar) media tilted. Then incubated at 37ºC for 24 hours in an incubator (Bakhtra *et al*., 2018).

Mc.Farland Standard Manufacturing

Enter 0.05 ml of 1.175% BaCl2.2H2O solution and mix it with 9.95 ml of 1% H2SO4 solution into a test tube, then shake until a cloudy solution is formed. This solution was used as a standard for test bacterial turbidity (Mayefis *et al.*, 2020*)*.

Manufacture of Negative Control Antibacterial Test

The negative control used in this study used a 10% DMSO solvent. Preparation of a 10% solution of Dimethyl sulfoxide (DMSO) was carried out by dissolving 10 ml of DMSO into 90 ml of aquadest (Putri *et al*., 2013).

Bacterial Suspension Manufacturing

Bacterial cultures that have been aged for 24 hours are taken from 2 oses of oblique agar, the test bacterial colonies are suspended in 10 ml of sterile 0.9% NaCl in a sterile test tube. Then homogenized with a vortex. Suspension turbidity was compared with Mc.Farland (Bakhtra et al., 2018).

Antibacterial Activity Test

Antibacterial testing was carried out using the paper disc diffusion method. Evenly scratch the bacterial suspension as much as 50 L using a sterile cotton and then leave it for 1-5 minutes so that the suspension is completely absorbed into the agar.

Place the disc paper on the watch glass, take 10 L of each fraction test solution on the disc paper, wait until the sample extract is completely diffused, then remove the disc paper

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using sterile tweezers and place the disc paper on the NA medium. As a negative control, DMSO 10% was used and a positive control was used Tetracycline paper disk. This treatment was repeated three times. Then these petri dishes were incubated in an incubator for 24 hours at 37 C. Then the antibacterial activity was determined by measuring the diameter of the inhibition area formed using a caliper (Hidayat et al., 2023)

Data analysis

Data analysis in this study was descriptive data with data presentation and also in tabular form by observing the diameter of the inhibition zone of the clear area of the extract of the Sea Grape Fraction.

RESULTS AND DISCUSSION

Sea grapes were extracted by maceration method by immersing using 95% ethanol solvent because 95% ethanol solvent is a high concentration that can attract chemical content in the sample well and ethanol solvent is also low in toxicity, so that the chemical content in the sample is not damaged. The maceration method is used because maceration is an extraction method that is technically working (Ritan *et al*., 2021).

The maceration method is used because maceration is an extraction method that technically works and the tools used are simple, that is, it is enough to soak the sample in organic solvent for 3-5 days with occasional stirring.

From the 20 kg sample of sea grapes, it was obtained that the thick ethanol extract was 425 grams with a yield of 2.12%. The calculation of the yield value serves to determine how many samples are needed for extraction in order to obtain the desired number of extracts.

The thick extract of Sea Grape (*Caulerpa racemosa*) obtained was divided by 2. The extract used for fractionation was 213 grams while the extract was 212 grams. Fractionation aims to separate compounds based on their level of polarity. Non-polar compounds tend to dissolve in non-polar solvents while polar compounds tend to dissolve in polar solvents. Solvent n-hexane is a non-polar solvent used to dissolve non-polar compounds such as oils, carotenoids, steroids and terpenoids. Meanwhile, semi-polar solvents such as ethyl acetate can dissolve aglycone flavonoid compounds. From the fractionation process, the weight of n-hexane fraction was 1.705 grams and Ethyl acetate was 3.115 grams.

In this study, based on phytochemical screening, the secondary metabolites in the Ethyl acetate fraction of Sea Grape (*Caulerpa racemosa*) were alkaloids, flavonoids and saponins, while the n-hexane fraction of Sea Grape showed negative results for secondary metabolites of alkaloids, flavonoids, saponins, phenolics. , terpenoids (Table1)

Alkaloids act as antibacterial compounds. Caulerpin compounds found in sea grapes produce secondary metabolites, namely bisindole alkaloids, bisindole compounds are found to be strong bioactive compounds, some of which include antifungals, and antibacterial .

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Flavonoids have antibacterial activity by interfering with metabolic function by destroying cell walls and denaturing bacterial proteins (Zhou et al., 2023).

While saponins have antibacterial activity by disrupting the surface of the cell wall. When disturbed, antibacterial substances will easily enter bacterial cells (Hayati et al., 2023)

The method used in the antibacterial test of the fraction of Sea Grape extract was the diffusion method using disc paper. The diffusion method was chosen because this method can clearly observe the presence or absence of bacterial growth so that it can facilitate the observation of the test bacteria. This inhibition of bacterial growth is characterized by the presence of a clear zone around the disc, the formation of a clear zone around the disc is caused because in that area the growth of bacteria is inhibited by the test sample.

In this study using a positive control Tetracycline. The tetracycline group includes antibiotics that are bacteriostatic. Tetracycline is a broad-spectrum antibiotic that can fight a large number of Gram-positive and Gram-negative bacteria While DMSO was used as a negative control because it did not affect the results of observations and did not provide activity against the growth of bacteria or fungi (Pelo et al., 2020). In research, the results of the antibacterial exract test in table 2 until table 3.

Table 1. Results of Phytochemical Screening Test for the Ethyl Acetate Fraction of Sea Grape (*Caulerpa racemosa*)

No	Parameters	Of reagents	Fraction ethyl aceatate	n-hexane	extract
1.	Alkaloids	Mayers reagent	(+) white precipitate	(-) Negative	(+) white precipitate
2.	Flavonoid	Powder Mg	(+) dark red color	(-) Negative	(+) dark red color
3.	Saponins	Hot aquadest and concentrated hcl	(+) foam permanent	(-) Negative	(+) foam permanent
4.	Phenolic	FeCl3	(-) Negative	(-) Negative	(-) Negative
5.	Terpenoids	Sulfuric acid and acetic acid	(-) Negative	(-) Negative	(-) Negative

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Table 2. The results of the antibacterial activity of the Sea Grape extract against bacteria *Klebsiella pneumoniae*

Treatment	Bacteria Inhibition (mm)			
	Treatment 1	Treatment 2	Treatment 3	Average
Concentration 25 %	6,9 mm	7,1 mm	7,2 mm	7 mm
Concentration 75 %	8,5 mm	9,1mm	9,1 mm	9 mm
Concentration 100 %	12,4mm	12,1 mm	11,8 mm	12,1 mm
Control (+)(Tetracycline)	23,8 mm	22,5 mm	23,1 mm	23,1 mm
Control (–) (DMSO 10%)	-	-	-	-

Table 3. The results of the antibacterial activity of the Sea Grape extract against bacteria *Micrococcus luteus*

Treatment	Bacteria Inhibition (mm)			
	Treatmen 1	Treatment 2	Treatment 3	Average
Concentration 25 %	7,5 mm	7,1 mm	6,7 mm	7,1 mm
Concentration 75 %	14,5 mm	14,1 mm	13,6 mm	14 mm
Concentration 100 %	16,8 mm	16,1 mm	17,2 mm	16,7 mm
Control (+)(Tetracycline)	25,4 mm	25,6 mm	23,2 mm	24,7 mm
Control (–) (DMSO 10%)	-	-	-	-

Antibacterial activity can be measured by the diameter of the inhibition zone which can be grouped into 4 groups, namely: the diameter of the inhibition zone <5 mm is categorized as weak, the diameter of the inhibition zone is 5-10 mm is categorized as moderate, the diameter of the inhibition zone is 10-20 mm is categorized as strong, and the diameter of the inhibition zone is >20 mm is categorized as very strong (Greenwood, 1995).

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CONCLUSION

Sea Grape (*Caulerpa racemosa*) extract had antibacterial activity against *micrococcus luteus* and *Klebsiella pneumoniae* while Sea Grape (*Caulerpa racemosa*).

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