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Antibacterial Effectiveness Test of Methanil Extract of Brown Algae (Sargassum vulgare) Against Staphylococcus aureus Bacteria

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ABSTRACT
Infectious diseases are one of the main causes of morbidity and mortality globally, especially in developing countries. One of the most common and dangerous pathogenic bacteria is Staphylococcus aureus, that can cause a range of infections by mild skin problems to life-threatening diseases. This study aimed to evaluate the antibacterial activity of methanol extract of brown algae Sargassum vulgare against S. aureus. This laboratory-based experimental research was conducted. Extraction was carried out utilizing the maceration technique with methanol solvent. Phytochemical screening was performed to detect alkaloids, flavonoids, saponins, and steroids. Antibacterial activity was tested utilizing the disk diffusion technique at extract concentrations of 5%, 10%, and 15%, with chloramphenicol as the positive control and aqua pro injection as the negative control. The outcomes performed that the extract contained alkaloids, flavonoids, and saponins, but not steroids. However, no inhibition zone was observed at any extract concentration against S. aureus. In conclusion, although the extract contained bioactive compounds, it did not perform antibacterial effectiveness against *S. aureus*.

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INTRODUCTION

Indonesia's biological resources are very abundant including brown algae diversity that has not been studied in depth so that the potential of brown algae has not been widely explored in the field of health. Brown algae (S. vulgare) contains bioactive compounds such as flavonoids, alkaloids, saponins and steroids drawn as antibacterial activity. The (Pangestuti et al., 2017).first step is to introduce brown algae as an alternative to natural antibacterial drugs and can be an alternative because it has minimal side effects

Infectious diseases are one of the leading causes of morbidity and mortality worldwide, especially in developing countries. Bacterial infections remain a serious challenge in the global public health sector due to their rapid spread and systemic impact. One of the most common and dangerous pathogenic bacteria is Staphylococcus aureus, that is drawn to cause a variety of diseases ranging by mild skin infections to severe diseases that can be life-threatening (Bitrus et al., 2018).

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In Indonesia, S. aureus is commonly found as a cause of skin infections, respiratory tract infections, and wound infections characterized by pus formation due to its pyogenic nature (Indira et al., 2024). These bacteria are able to survive on various surfaces and dry environments, and produce toxins such as leukocidin and enterotoxins that aggravate the symptoms of infection (Anjani, 2024). Treatment of infections caused by S. aureus is generally done with antibiotics such as chloramphenicol, methicillin, and penicillin. However, long-term excessive and inappropriate use of antibiotics has led to the emergence of antibiotic resistance, that poses a serious threat to public health (Hilmi et al., 2018; Pratiwi, 2017).

To overcome the problem of antibiotic resistance, alternative approaches through the utilization of natural materials as antibacterial agents continue to be developed. One potential biological source is the brown alga Sargassum vulgare, that is drawn to contain various bioactive compounds such as alkaloids, flavonoids, saponins, and steroids that play a role in antimicrobial activity (Pangestuti et al., 2017; Sampulawa et al., 2022). (S. vulgar) is found in many coastal areas of Indonesia, including in Papuan waters, but until now it has not been optimally utilized by the community, and is still considered a wild plant (Pakidi et al., 2016).

Indonesia's biological resources are very abundant including brown algae diversity that has not been studied in depth so that the potential of brown algae has not been widely explored in the field of health. The (Pangestuti et al., 2017).first step is to introduce brown algae as an alternative to natural antibacterial drugs and can be an alternative because it has minimal side effects

As a maritime country with abundant biological wealth, Indonesia has a great opportunity to develop natural resources as traditional medicinal materials, especially in supporting national health independence. The utilization of S. vulgare as an antibacterial agent not only contributes to infection control, but also becomes a sustainable solution to reduce dependence on synthetic antibiotics that have side effects and high risk of resistance (Rizkaprilisa et al., 2023; Magvirah et al., 2020).

Based on this background, this study aims to evaluate the antibacterial activity of brown algae extract Sargassum vulgare against Staphylococcus aureus bacteria, as an effort to develop natural ingredients that have the potential to be an alternative treatment for infections caused by pathogenic bacteria..

METHODS

This research is a type of laboratory experimental quantitative research that aims to test the antibacterial activity of methanol extracts by brown algae (Sargassum vulgare) against Staphylococcus aureus. The research was conducted at the Natural Materials Laboratory and Microbiology Laboratory, Pharmacy Study Program, Faculty of Applied Science, Muhammadiyah Sorong University of Education, by November 2024 to January 2025.

Tools and Materials

The tools used include autoclave, aluminum foil, sieve, stir bar, bunsen, blander, glass bottle, petri dish, funnel, erlenmeyer (Pyrex), beaker (Pyrex), measuring cup, handscoon, hot plate, incubator, ose needle, vernier caliper, filter paper, disc paper, label paper, lighter, laminar air flaw, micropipette, dropper pipette, plasti wrap, test tube rack, marker, spatula,

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spirtus, stamper, sudip, mattress strap, test tube, jar, analytical balance, and test tube, and water bath.

The materials used include chloramphenicol antibiotic 2 µg disk, pro injection distilled water, sterile distilled water, anhydrous acetic acid ($CH_3CO)2O$, sulfuric acid (H_2SO_4), hydrochloric acid (HCL 2N), bacteria S. aureus $BaCl_2$1% (barium chloride), iron (III) chloride ($FeCl_3$), brown algae (S. vulgare), NaCl 0.9% (sodium chloride), Pb II acetate, mayer reagent, dragendroff reagent, and bauchardat reagent.

Working Procedure

The sample used is brown algae as much as 1100 gr by the Galilee cape of southwest papua. Brown algae are cleaned with running water, drained and then chopped and dried utilizing an oven at 40 ° C. the outcomes of drying brown algae are pulverized utilizing a blender to produce powdered simplisia.

Sample extraction utilizing maceration technique with methanol solvent. 514 grams of brown algae simplisia powder was put into a glass jar and soaked in methanol solvent as much as 2000 mL for 3 days. Continued remaceration with 2000 mL of solvent for 3 days. The outcomes of the macerate were then evaporated utilizing a waterbath to obtain a thick extract of brown algae.

Phytochemical Screening

Phytochemical tests were carried out to determine the presence of compound class content in brown algae test samples. Flavonoid test A total of 2 mL of brown algae extract (S.vulgare) was put in a test tube and added in 1-2 mL of Pb II acetate. The presence of flavonoid compounds is characterized by the formation of yellow sediment (Arni et al., 2024).

Alkaloid test utilizing mayer reagent as much as 2 mL of extract added mayer reagent as much as 2 drops. The presence of alkaloid compounds in the mayer reagent is characterized by the formation of a white or yellow precipitate. Alkaloid test utilizing bouchardat reagent, 2 mL of extract added 2-3 drops of bouchardat reagent. The presence of alkaloid compounds in the bouchardat reagent forms a black brown precipitate. Saponin test utilizing HCl solution, 2 mL extract and 2 mL HCl solution then dikosock for 1 minute. if foam is formed then the extract contains saponin. Test steroid extract sebnayk 2 mL plus reagent anhydrous acetic acid 2 drops and sulfuric acid 1 drop. The presence of steroid compounds is marked by the formation of a bluish green color.

Sterilization of tools is carried out utilizing an autoclave at 121 ° C for 15 minutes. tools that have been washed and dried are wrapped one by one utilizing clean paper and then tied utilizing a rope. Concentration test solutions were made with concentrations of 5%, 10% and 15% by weighing 0.5 g, 1 g, and 1.5 g of brown algae extract each dissolved with aqua pro injection to a volume of 10 mL. The positive control used chloramphenicol disk. Negative control aqua pro injection

The nutrient agar media of 7 g was dissolved with aqua pro injection as much as 250 mL and then homogenized with a magnetic stirrer on a hotplate until it turned clear. Nutriet agar that has been homogenized is sterilized utilizing an autoclave at 121 ° C for 15 minutes.

Saureus bacteria were cultured by scraping with an ose needle on a slanted agar medium aseptically. The tube was incubated for 24 hours at 37°C. One ose of rejuvenated

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bacteria was inoculated into a test tube containing 10 mL of 0.9% NaCl solution and homogenized until it reached the turbidity of Mc farland bacterial suspension.

Antibacterial Activity Test

Antibacterial activity testing was carried out by the agar diffusion technique utilizing discs. Nutriet agar was poured into each Petri dish and allowed to solidify. Bacterial culture solution was scraped onto the Petri dish in a zigzag manner utilizing a cotton swab. Sterile disks that have been soaked in the concentration solution are transferred utilizing sterile tweezers to the top of the agar medium. After the discs of 5%, 10% and 15% concentration, positive control and negative control were inserted into the petri dish then incubated for 24 hours at 37°C.

Observation and Measurement

The diameter of the inhibition zone around the disk after incubation for 24 hours can be observed utilizing a push-pull term. Data by the measurement of the diameter of the inhibition zone were statistically analyzed utilizing the One-way ANOVA test with the help of SPSS software version 25 at a significance level of 5% to compare treatments between extract concentrations against the growth of S. aureus bacteria.

RESULTS AND DISCUSSION

This research consists of three stages of outcomes, namely extract yield, phytochemical screening test, and antibacterial effectiveness test of brown algae extract (Sargassum vulgare).

Table 1. Yield of Brown Algae Extract (S. vulgare)

Simplisia	Sample Weight (gram)	Powder Weight (gram)	Ectract Weight (gram)	Yield (%)
Brown algae	1100	514	29	5,6%

Source: Primary Data, 2025

The initial weight of brown algae simplisia was 1100 grams and then dried utilizing an oven at 40°C to obtain a dry powder of 514 grams. The dry powder was extracted by maceration technique for 3 days and continued remaceration for 3 days. The extract was then evaporated utilizing a 40°C waterbath, outcomeing in an extract weight of 29 grams. Based on the calculation, the yield fulfilled was 5.6%.

Phytochemical Screening outcomes of Brown Algae Extract (S. vulgare)

Table 2. Phytochemical Screening outcomes of Brown Algae Extract (S. vulgare)

Group of compounds	Reagent	Observation outcome	Test outcome
Alkaloid	Mayer	Reacts white or yellow	+
	Bouchardat	Dark brown precipitate	+
Flavonoid	Pb(II) asetat	Yellow precipitate	+
Saponin	HCl 2N	There is froth	+
Steroid	Liebermann–Burchard	No bluish green color	-

Source: Primary Data, 2025

Description: (+) Contains secondary metabolite compounds, (-) Does not contain secondary metabolite compounds.

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Phytochemical screening was carried out to detect the content of secondary metabolite compounds in brown algae extract. Based on the outcomes in Table 2, it is drawn that brown algae extract (S. vulgare) contains alkaloid, flavonoid, and saponin compounds, but does not contain steroids. Alkaloid tests utilizing Mayer's reagent performed positive outcomes with the formation of a white or yellow precipitate, and Bouchardat's reagent performed a black brown precipitate. This reveals the presence of alkaloid compounds containing nitrogen atoms with free electron pairs that can form complexes with metal ions (Tanfil T. et al., 2023).

Flavonoids were tested with Pb(II) acetate that produced a yellow precipitate, indicating the content of flavonoids that have the potential to inhibit bacterial metabolism (Suryatman & Achmad, 2022). Saponins performed positive outcomes with the formation of foam when shaken, indicating the presence of surfactant properties that reduce surface tension (Ravelliani et al., 2021; Anggraeni Putri et al., 2023). Conversely, the steroid test performed negative outcomes because no bluish green color was formed, indicating the absence of steroid compounds in the extract (Arni et al., 2024).

Table 3. outcomes of Antibacterial Effectiveness Test of Brown Algae Methanol Extract (S. vulgare) against Staphylococcus aureus Bacteria

Concentration (%)	Replica 1	Replica 2	Replica 3	Average (mm)
5%	0 mm	0 mm	0 mm	0 mm
10%	0 mm	0 mm	0 mm	0 mm
15%	0 mm	0 mm	0 mm	0 mm
Negative control (aqua pro injection)	0 mm	0 mm	0 mm	0 mm
Positive control (disk kloramfenikol 2 µg)	18,8 mm	20 mm	18,5 mm	19 mm

Source: Primary Data, 2025

The antibacterial effectiveness test utilizing the disc diffusion technique performed that brown algae extract at concentrations of 5%, 10%, and 15% did not cause an inhibition zone against Staphylococcus aureus bacteria, while the positive control (chloramphenicol) produced an average inhibition zone of 19 mm. This reveals that the methanol extract of brown algae (S. vulgare) has no antibacterial activity against S. aureus.

This outcome is different by prior research by Indria et al. who fulfilled an inhibition zone at the same concentration. This difference can be caused by various factors such as the type of bacterial strain, the geographical situations where the algae grow, and the possibility of degradation of active compounds during the extraction and storage procedure (Pangestuti et al., 2017; Putri, 2021; Rishliani, 2022).

A decrease in antibacterial activity is also possible due to the degradation of secondary metabolite compounds during the drying and heating procedure, as well as uncertainty in the amount and type of antibacterial compounds contained (Sofia et al., 2023). Thus, although the extract performed the content of bioactive compounds, its antibacterial effectiveness against S. aureus has not been proven in this study.

The yield outcomes by the extraction procedure of brown algae (Sargassum vulgare) perform a fairly low amount of extract compared to the initial weight of the simplisia. This

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yield value reflects the efficiency of the extraction technique and the content of soluble compounds in methanol solvent. The greater the yield fulfilled, the more secondary metabolite content that is successfully withdrawn by the raw material (Sofia et al., 2023).

The low yield in this study could be influenced by the extraction technique used, that is maceration. Although this technique is often chosen for its simplicity, its efficiency is not always high because it only relies on passive movement of molecules. The diffusion speed of active compounds into the solvent can be limited by the structure of plant tissues that are still dense (Yainahu et al., 2023).

The drying temperature factor also affects the yield. Drying at 40°C does aim to maintain the stability of active compounds, but on the other hand it can slow down the dehydration procedure and cause the growth of microorganisms that damage bioactive compounds. If this procedure is not optimal, the active compounds can be degraded before the extraction procedure takes place (Maslahah, 2024).

In addition, solvent selection plays an important role in determining the amount of yield. Methanol is a polar solvent drawn to dissolve compounds such as alkaloids, flavonoids, and saponins, but not all compounds are polar. If there are non-polar compounds in brown algae, then these compounds cannot be extracted completely (Yuliarni et al., 2022).

The outcomes of phytochemical screening performed that the methanol extract of S. vulgare contained alkaloids, flavonoids, and saponins, but no steroid compounds were found. The presence of these secondary metabolite compounds reveals that brown algae do have ability as active ingredients for various pharmacological applications, including antibacterial, antioxidant, and anti-inflammatory (Fitrah 2022).

Alkaloids are nitrogenous base compounds that are often associated with high biological activity. These compounds are drawn to have the ability to interfere with the physiological functions of microorganisms, especially in protein synthesis and energy metabolism. The presence of alkaloids in the extract gives an indication that S. vulgare has potential bioactivity against pathogenic microbes (Tanfil. T et al., 2023).

The flavonoids in the extract also reinforce this potential. These compounds are drawn to inhibit bacterial growth by attacking the cell wall structure and disrupting important enzymatic pathways in the bacterial body. Some prior studies stated that flavonoids act as natural antibacterial agents that are effective against gram-positive and gram-negative bacteria (Swantara et al., 2022).

Saponins found in the extract also contribute to the bioactivity of the extract. Saponins have the ability to lower surface tension and are surfactant-like. This mechanism allows saponins to destroy bacterial cell membranes, cautilizing leakage of cell contents and ultimately bacterial cell death (Anggraeni Putri et al., 2023).

Conversely, the non-detection of steroid compounds in the extract can be caused by several possibilities. One of them is because the steroid content in S. vulgare is indeed very low or below the detection limit of the test technique used. Another possibility is that the compound is degraded during the drying or extraction procedure (Maryam et al., 2020).

The absence of these steroid compounds can also be attributed to variations in the environmental situations in that the algae grow. According to prior studies, the production of

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secondary metabolites in marine organisms is strongly influenced by external factors such as salinity, light intensity, and water temperature. These variations can cause differences in the content of bioactive compounds in the same species by different locations.

Although S. vulgare extract contains bioactive compounds that theoretically have antibacterial activity, the test outcomes against Staphylococcus aureus performed no inhibition zone. This reveals that these compounds have not been able to perform biological effectiveness at the concentrations and test situations used (Pangestuti et al., 2017).

Several factors can explain the lack of antibacterial activity. One of them is that the concentration of active compounds is too low. The metabolite compounds contained in the extract may not have reached effective levels to inhibit the growth of S. aureus, that is drawn as a gram-positive bacterium with thick cell walls and is resistant to various natural antibacterial compounds (Putri, 2021).

Another factor is the possible degradation of active compounds during the extraction or storage procedure. Certain flavonoid and alkaloid compounds are very sensitive to temperature, light and oxygen. If degradation occurs, the antibacterial potential of the compound may decrease significantly (Veny et al., 2024).

The difference in outcomes with prior studies can also be attributed to the variety of S. aureus strains used. Each strain has different biochemical and genetic characteristics that can respond to antibacterial compounds with varying levels of resistance. Resistant strains of test bacteria can lead to negative outcomes even though the active compound is actually present in the extract (Riyanto et al., 2023).

In addition, the location of algae sampling also contributes to differences in secondary metabolite content. Algae that grow in environments with high sun exposure or exposure to certain pollutants tend to produce more bioactive compounds as a self-defense response. In contrast, algae by stable environments tend to have lower compound content (Rishliani, 2022).

The lack of antibacterial effectiveness can also be attributed to the possibility that the bioactive compounds in the extracts are not yet in pure form. A mixture of metabolites that have not been isolated may not provide a strong synergistic effect against bacteria. The purification procedure can increase the concentration of certain compounds and open up opportunities to perform more pronounced antibacterial activity (Sampulawa et al., 2002).

Finally, the chemical structure of the compound also affects its biological effectiveness. Although compounds such as flavonoids and alkaloids are found, if their molecular structures do not have the right functional groups or are unable to interact with the target molecules inside the bacterial cells, no significant antibacterial activity will occur. This reinforces the importance of structural characterization of compounds in follow-up studies (Indira & Mariadi, 2024).

Thus, although brown algae performs good phytochemical potential, its effectiveness as an antibacterial agent has not been strongly proven against S. aureus. Further research with a focus on isolation of active compounds, testing against different types of bacteria, and variation of extraction techniques is highly recommended so that the therapeutic potential of S. vulgare can be optimized.

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CONCLUSION

Based on the outcomes of the research that has been done, it can be concluded that brown algae extract (Sargassum vulgare) performs the presence of secondary metabolite compounds in the form of flavonoids, alkaloids, and saponins based on phytochemical screening tests. However, the extract did not perform activity as an antibacterial against the growth of Staphylococcus aureus bacteria. Based on these findings, it is recommended that further research be carried out quantitatively utilizing spectrophotometric techniques to determine the levels of bioactive compounds contained in brown algae methanol extracts and isolate active compounds to identify antibacterial potential more specifically against S. aureus and other microorganisms.

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