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Potential Antimicrobial Ethyl Acetate Extracts of Ur Burst Shells Against S.aureus Bacteria From Diabetic Foot Wounds

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Abstract: Diabetes mellitus (DM) is a global problem that requires special treatment. One of the complications of DM is diabetic foot ulcers. The spread of diabetic wounds accompanied by ulcers is caused by the presence of S. aureus bacteria. This bacterium is known to be able to adapt to various classes of antibiotics, so it is necessary to explore new antibiotics from natural materials, one of which is sea urchins. This study was conducted to determine the antibacterial activity of ethyl acetate extract of sea urchin shells on the growth of S. aureus bacteria. This research was conducted at the Laboratory of the Medica Farma Husada Polytechnic in Mataram and the URB Laboratory of the NTB Provincial Hospital. The results of this study showed that there was the antibacterial activity of sea urchin shell extract with an inhibition zone diameter of 12 mm which was classified as strong, while Ciprofloxacin formed an inhibition zone with a diameter of 27 mm. From this study, it was concluded that sea urchin shell extract was able to inhibit the growth of S. aureus.

Keywords: Diabetes Mellitus; *S. aureus*; Diadema setosum shell extract; antibiotic

Introduction

A disease that is a global health problem with a significant increase every year is diabetes mellitus (DM) (Shaw et al., 2010). Indonesia is a country with a high prevalence of DM. Indonesia is included in the 10 countries with an increasing prevalence of DM (IDF, 2017).

One of the manifestations of diabetes complications is diabetic foot ulcers (LKD). The global prevalence of DFS is estimated at 6.3% (Zhang et al., 2016). A study conducted in Indonesia stated that the prevalence of DLE was 12% (Blanes et al., 2011; Yusuf et al., 2016; Wahyuningrum et al., 2020).

Infection in diabetic ulcers is one of the aggravating factors which is also a benchmark for therapy and management that must be given. In immunocompromised patients, DLE can worsen, supported also by the presence of microbes that are resistant to antibiotics. Some of the microbes present in LKD ulcers often require specific antibiotics which are expensive and prolonged (Langi, 2013).

Based on several studies, the bacteria that are often found in diabetic ulcers are Klebsiella sp, Proteus mirabilis, Staphylococcus epidermidis, Staphylococcus aureus, Enterobacter sp, Proteus Vulgaris, Pseudomonas aeruginosa, and Escherichia coli. (Risnawati, Saldy Yusuf, 2018; Decroli, 2018; Akhter et al., 2012).

According to several studies in Indonesia, Staphylococcus aureus is one of the most common bacteria found in diabetic ulcers. Staphylococcus aureus is a pathogen that plays a role in progressive and widespread tissue destruction and is accompanied by purulent abscesses (Khariunnisa et al., 2020). Staphylococcus aureus tends to have the ability to adapt to antibiotics so that it is resistant to several antibiotics (Shahi et al., 2013). A recent study suggested that S.

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aureus obtained from diabetic foot ulcers was resistant to amoxicillin (Khariunnisa et al., 2020).

The increasing resistance of bacteria to antibiotics causes an urgency to search for new antibacterial agents from various sources, including natural sources. Marine invertebrates usually rely solely on innate immune mechanisms with a predominance of cellular components. This cellular component is characterized by hemocytes which will phagocytize microbes and secrete antimicrobial substances and are soluble and cytotoxic. Thus, marine invertebrates become a potential source of promising antibacterial compounds with new mechanisms of action (Abubakar et al., 2012).

Indonesia is an archipelagic country with potential marine resources to be utilized in a sustainable manner (Lasabuda, 2013). West Nusa Tenggara is a province with an exotic coastline and diverse resources and its potential as a medicinal ingredient can be explored. One of the marine biotas with potential as a medicinal ingredient is the sea urchin (Diadema stetosum) (Arthaz, 2015; Yusron, 2010; Maleta et al., 2018).

Sea urchins are often found on the coastline of the West Lombok region, especially around the coast of the Sekotong area. The sea urchin shell has a hard texture and is coated with a stable black pigment. Polyhydroxy and apelasteroids A and B are known to be abundant in sea urchin shells (Baransano & Mangimbulude, 2018). Based on previous research, it is known that the antimicrobial and antioxidant compounds in sea urchins are quite high with an IC50 value of 1,451 ppm (methanol extract) (Olivia Akaerina et al., 2015).

The active compounds that are toxic in sea urchin shells are thought to be used as medicinal ingredients. The active substances contained in sea urchin shells include steroids, glycosides, serotonin, cholinergic substances, and bradykinin-like substances (Pranoto et al., 2012). Antibacterial activity of ethyl acetate extract of sea urchin shells on the growth of Staphylococcus aureus bacteria is what will be seen in this study, with the test parameter being the inhibition zone formed. This study need to be done to get a novel information about this extract as an alternative antibiotic to *S. aureus* and will be developed as a new treatment for ulkus diabeticum.

Method

This study used a laboratory experimental design to observe the antibacterial activity of ethyl acetate extract of sea urchin shells on the growth of Staphylococcus aureus bacteria. This research has obtained ethical approval with the number 248/UNI18.F7/ETIK/2022 and was conducted at the Laboratory of the Medica Farma Husada Polytechnic in Mataram and the Laboratory of the Biomedical Research Unit (URB) RSUDP NTB.

The sample used in this study was sea urchin shells obtained from fishermen around Ekas beach, East Lombok. The S.aureus bacterial isolate used was obtained from diabetic foot ulcers of patients and purified at the URB Laboratory of RSUDP NTB.

Tools and materials

The tools used in this research were an oven, blender, knife, rotary evaporator, mortar, stamper, pH meter, transparent glass, glassware, micropipette, tip, caliper, loop, stir bar, filter paper, petridish cup, aluminum cup, incubator, glassware, filter, centrifuge, spectrophotometer, refrigerator, laminar air flow, vortex, plastic tube, tip, and micropipette. The main ingredient in the research is sea urchin shell extract. Bacterial isolates of Staphylococcus aureus, Ciprofloxacin, aqua dest, and Ethyl acetate (p.a).

Preparation of Sea Urchin Shell Extract

Air-dried sea urchin shells are blended/grinded into powder. The powder is sieved using 100 mesh to obtain a finer powder. The resulting powder was then soaked in ethyl acetate (1:3) for 7 days with daily stirring. Once every 2 days, the extract is filtered using Whatman filter paper, until on the 7th day a final filtering is carried out then evaporation is carried out to obtain a thick extract.

Making Muller Hinton Agar (MHA) Media

0.95 grams of MHA media was dissolved in 50 ml of distilled water (pH 7.4). Once dissolved, transfer the media to an Erlenmeyer tube to be sterilized at 1210C in an autoclave. Sterile media is poured into the petri dish aseptically to the appropriate thickness.

Preparation of Bacterial Suspensions

A pure isolate of Staphylococcus aureus was prepared and then taken using a sterile round loop and suspended with 1 ml of 0.9% NaCl in a test tube.

Testing the Antibacterial Activity of Sea Urchin Shell Extract

Prepare tools and materials. A sterile cotton swab was dipped into the bacterial suspension and aseptically spread into the MHA medium. Media sections are marked and divided into areas using markers. Leave it for about 5-10 minutes so that the bacterial suspension diffuses properly in the media. Wells were made in MHA media (cork borer) to place extracts and antibiotics. Enter the extract and antibiotics into the wells that have been prepared. Incubated for 1x24 hours and measured the inhibition zone.

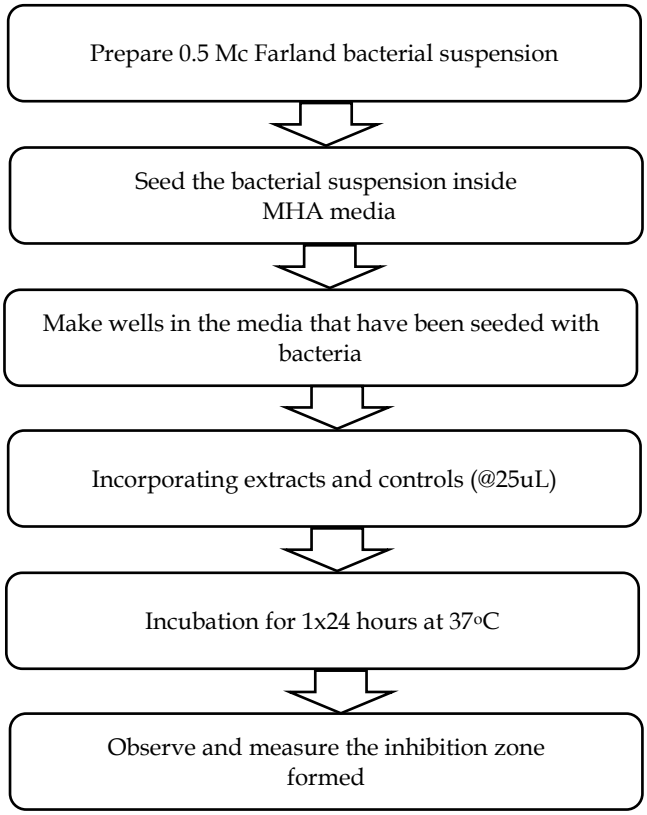

Figure 1. Flowchart of extract sensitivity test

Result and Discussion

This study tested the antibacterial activity of sea urchin shell extract (ethyl acetate) on the growth of S.aureus isolates of diabetic foot ulcers in patients with ciprofloxacin as a control. The results obtained showed the presence of antibacterial activity with a strong level of bacterial sensitivity (Table 2). The inhibition category in terms of the diameter of the inhibition zone formed is presented in Table 1.

Table 1. The inhibition category in terms of the diameter of the inhibition zone

Diameter	Growth inhibition response
<5mm	Weak
6-10mm	Currently
11-20mm	Strong
>21mm	Very strong

Source: (Bhargav et al., 2016)

From Table 2 it is known that the concentration zones of the extracts used were 5%, 10%, 25%, and 45%. The inhibition zone was only formed at concentrations of 5 and 10% with an average diameter of 12 mm (Strong), while the positive control was 27 mm (Very strong). El-Sayed et al. (2020), stated that the crude extract of sea urchins had antibacterial activity for gram-positive bacteria but not for gram-negative bacteria. This is caused by differences in the structure of the cell membrane of gram-positive and gram-negative bacteria. The inhibition zone formed on the media with S.aureus bacteria was 6.5 mm. this diameter is half of the diameter formed in our study. The results of this study are quite different from our research. This is probably because the extract we used was shell extract whereas previous studies used crude extract or the whole sea urchin itself.

Table 2. Results of sensitivity test of sea urchin shell extract on the growth of S. aureus.

Repetition	Extract a concentration of 5%	Extract a concentration of 10%	Extract concentration of 25%	Extract concentration of 45%	Clear zone (mm) C+	C-
1	12	12	0	0	27	0
2	12	12	0	0	27	0
3	12	12	0	0	27	0

Research result El-Sayed et al. (2020), is almost similar to those (Rompas et al., 2022), where the ethyl acetate extract from sea urchins as a whole has antibacterial activity against the growth of S. aureus with an inhibition zone diameter of 7.67 mm, while in the study Indrawati et al. (2018), the inhibition zone formed with sea urchin gonad extract was 14 mm. In line with previous studies, ethyl acetate extract from sea urchin shells is known to inhibit the growth of S. aureus. This is a manifestation of the antibacterial content in sea urchin shells in the form of flavonoids, alkaloids, and tannins (Pelu et al., 2020; Apriandi et al., 2020; Rompas et al., 2022).

Flavonoids as antibacterial have a mechanism of action forming bonds with bacterial extracellular proteins which will eventually damage the bacterial cell membrane (Amalia et al., 2017). Meanwhile, the toxicity of tannins is one of the causes of damage to the bacterial metabolic system so that the living function of the bacteria becomes disrupted (Chung et al., 2006). Diabetes mellitus (DM) is a degenerative disease with the main characteristic of hyperglycemia (Rahmasari & Wahyuni, 2019). One of the complications of DM is the presence of diabetic ulcers. Diabetic ulcers can then expand and become chronic when infected by bacteria with the ability to adapt to antibiotics. S. aureus plays a role in expanding wounds and causing ulcers (Khariunnisa et al., 2020; Cong et al., 2020).

Conclusion

Based on the research that has been done, it can be concluded that there is an antimicrobial activity of sea urchin shell extract on the growth of S. aureus as indicated by the formation of an inhibition zone around the extract. The limitation of this study is that the environmental conditions during the drying process to obtain a thick extract cannot be controlled optimally. So, the suggestion for further research is to perform an extraction with a variety of environmental conditions to obtain maximum extract results.

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