ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF SEA URCHIN SHELL (DIADEMA SETOSUM) AGAINST S. AUREUS BACTERIA FROM DIABETIC FOOT WOUNDS

Ajeng Dian Pertiwi[1], Hardani[2*], Dhika Juliana Sukmana[3], Bustanul Atfal[4]

1,2 Department Pharmacy, Politeknik Medica Farma Husada Mataram, Indonesia

3,4 Department of Medical Laboratory Technology, Politeknik Medica Farma Husada Mataram, Indonesia

Jl. Medica Farma No. 1, Baturinggit Selatan, Sekarbela, Mataram, Indonesia.

*Correspondence email: danylastchild07@gmail.com

Abstract: Diabetic ulcer is one form of chronic complication of diabetes mellitus in the form of open wounds on the skin surface that can occur due to infection caused by a bacterium from around the skin. Bacteria can produce biofilms and are resistant to antibiotics, so it is necessary to develop drugs for diabetic ulcer. One of them is the utilization of sea urchin shell (Diadema setosum) as an antimicrobial. Sea urchin shells contain bioactive compounds including serotoins, glycosides, steroids, cholinergic materials, and brandykinin-like substances (Dahl Jebson & Louis, 2010). Sea urchin shell extract has antibacterial effects on Eschericia coli, Salmonella sp. and Bacillus cereus using methanol solvent, ethyl acetate solvent and chloroform solvent (Hadinoto et al. 2017, Akerina et al. 2015). This study aims to determine the antibacterial activity of sea urchin shell methanol extract against the growth of S. aureus bacteria. This research was conducted at the Medica Farma Husada Mataram Polytechnic Laboratory and the URB Laboratory of the NTB Provincial Hospital. The results of this study showed that there was no antibacterial activity of sea urchin shell extract.

Keywords: Diabetes Mellitus; S. aureus; Sea urchin shell extract; Antibacterial.

1. Introduction

Diabetes mellitus is characterized by elevated blood glucose levels due to metabolic disorders. The pancreas in DM patients has a weakness in producing insulis hormone. This results in the distribution of blood glucose to other organs being disrupted so that blood glucose levels increase (Zychowska, M., et al. 2013). DM patients have a longer wound healing ability compared to normal humans. This is because wounds in DM conditions are included in chronic wounds due to the extension of the wound healing phase, namely haemostasis, inflammation, proliferation, and remodeling (Nagori B.P and Solanki R. 2011). Improper handling of chronic wounds with DM will result in infection which is generally treated with amputation (Karri, V.N.R., 2014). This causes people to look for alternatives in the treatment of diabetic wounds, one of which is by using herbal medicine. The use of herbal ingredients is increasingly favored by the public

with the back to nature trend. Many people use ingredients from natural materials, especially in preventive, promotive, and rehabilitative efforts to overcome various diseases (Mutiara, G. P.I., et al. 2015).

Diabetic ulcer is one of the chronic complications of diabetes mellitus in the form of open wounds on the skin surface that can occur due to infection caused by a bacterium from around the skin. Bacteria can produce biofilms and are resistant to antibiotics, so it is necessary to develop drugs for diabetic ulcers. One of them is the utilization of sea urchin shell (Diadema setosum) as an antimicrobial. Sea urchin shells contain bioactive compounds including serotoins, glycosides, steroids, cholinergic materials, and brandykinin-like substances (Dahl Jebson & Louis, 2010). Bioactive components detected in sea urchin gonads are steroids, triterpenoids and saponins. Sea urchin shell extract has antibacterial effect against Eschericia coli, Salmonella sp. and Bacillus cereus using methanol solvent, ethyl acetate solvent and chloroform solvent (Hadinoto et al. 2017, Akerina et al. 2015). The potential of sea urchin as an antibacterial needs to be developed because it can be utilized as a medicinal material in the pharmaceutical field. This study aims to obtain methanol extract of sea urchin shell and see the antibacterial activity against S.aureus bacteria.

2. Materials and Methods

This study used a laboratory experimental design to see the antibacterial activity of sea urchin shell methanol extract against the growth of Staphylococcus aureus bacteria. This research has obtained an ethical permit with number 248/UNI18.F7/ETIK/2022 and was conducted in the laboratory of Politeknik Medica Farma Husada Mataram and the Biomedical Research Unit Laboratory (URB) of RSUDP NTB.

The samples used in this study were sea urchin shells obtained from fishermen on Ekas beach, East Lombok. Isolate of S.Aureus bacteria 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 the research are Oven, Blender, Knife, Rotary evaporator, Mortar, Stamper, pH meter, transparent glass, glass tools, micropipette, tip, push term, ose, stirring rod, filter paper, petridish cup, aluminum cup, incubator, glass ware, filter, centrifuge, spectrophotometer, refrigerator, laminar air flow, vortex, plastic tube, tip, and micro pipette.

The main materials used in the study were sea urchin shell extract. Isolate of Staphylococcus aureus bacteria, Ciprofloxacin, aquadest and Methanol (p.a).

Preparation of Sea urchin Shell Extract

The dried sea urchin shells were blended/grinded into powder. The powder was then sieved using mesh 100. The resulting powder was soaked with methanol (1:3) for 7 days with daily stirring. Every 2 days, the extract was filtered using whatmann filter paper, until on the 7th day the final filtering was carried out and then evaporated to obtain a thick extract.

Preparation of Muller Hinton Agar (MHA) Media

Weighed as much as 0.95 g of MHA media then dissolved in 50ml of distilled water (pH 7.4). The solution was transferred to an Erlenmeyer tube to be sterilized at 1210C in an autoclave. Sterilized media was poured on Petri dishes aseptically to the appropriate thickness.

Preparation of Bacterial Suspension

Prepared pure isolate of Staphylococcus aureus then taken using a sterile round ose and suspended with 1 ml of 0.9% NaCl in a test tube.

Antibacterial Activity Testing of Sea urchin Shell Extracts

Tools and materials are prepared. Sterile cotton swabs were dipped in bacterial suspensions and spread into MHA media aseptically. Part of the media is marked and divided into several areas using a marker. Left for about 5-10 minutes so that the bacterial suspension diffused well on the media. Pits were made on MHA media (cork borer) to place extracts and antibiotics. Put the extracts and antibiotics into the prepared wells. Incubated for 1x24 hours then measured the clear zone/inhibition zone formed.

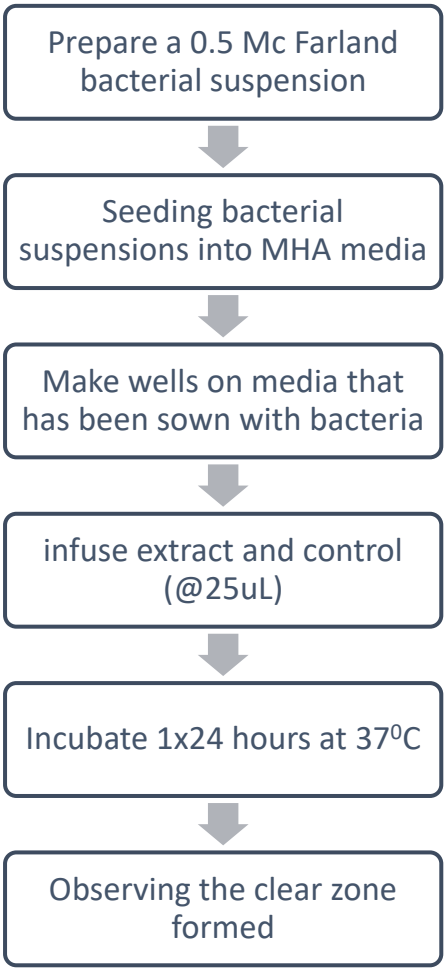

Figure 1 Flowchart of extract sensitivity test

3. Results and Discussion

In this study, the antibacterial activity of sea urchin shell methanol extract was tested against the growth of S. aureus bacteria isolated from foot ulcers of diabetic patients with negative control using Ciprofloxacin. The results obtained showed no antibacterial activity (Table 2). The category of inhibition is based on the diameter of the inhibition zone formed (Table 1).

Table 1. Inhibition category in terms of diameter of inhibition zone

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

Source: Stout in Fatimah, 2022

Table 2: Sensitivity test results of sea urchin shell extract against S.aureus growth.

Repeat	Zone of inhibition (mm)					
	Extract 5%	Extract 10%	Extract 25%	Extract 45%	C+	C-
1	0	0	0	0	30	0
2	0	0	0	0	30	0
3	0	0	0	0	30	0

In table 2, it can be seen that at all concentrations tested, no inhibition zone or clear zone was formed. One factor that affects the ability to inhibit bacteria from an extract is heating. The uncontrolled drying process carried out on the shell is thought to affect the inhibitory activity of the test bacteria. In general, bioactive components are quickly damaged because they are not heat-resistant (thermolabile) (Wang and Weller, 2006). These results are in line with research conducted by Akerina, 2015 where bacterial inhibition of methanol extract of sea urchin shells gave very small/weak results of 1 cm so that it can be categorized as insensitive. There are results of research by Aulia, 2020 where a large enough concentration of 80% is needed to be able to see the inhibition of methanol extracts against S.aureus.

4. Conclusion

Based on the research that has been done, it can be concluded that there is no antibacterial activity of sea urchin shell extract against the growth of S. aureus. The limitation in this study is that the environmental conditions during the drying process are still quite high and uncontrolled so that it can affect the results of the study. Suggestions for further research are to carry out extraction by controlling environmental conditions in terms of drying and testing at higher concentrations.

References

Akerina F.O, Tati .N, Ruddy. S., *Isolasi dan Karaakteristik Senyawa Antibakteri Dari Bulu Babi*. Jurnal Pengolahan Hasil Perikanan Indonesia IPB.

Dahl. W.J, Jebson. P. And Louis D.S.2010. *Sea Urchin Injuries to the Hand : A Case Report and Review of the Literature*. Iowa Orthopaedic Jounal 30.

Hadinoto S, Sukaryono D.I, Siahay Y. 2017. *Kandungan Gizi Gonad dan Aktvitas Antibakteri Ekstrak Cangkang Bulu Babi (Diadema setosum)*. Jurnal Pascapanen dan Bioteknologi Kementerian Kelautan dan Perikanan.

Karri V.N.R. 2014. *Current Perspective In The Management Of Diabetic Foot Ulcers-an overview on the indian scenario*. Internasional Journal of Pharmacy and Pharmaceutical Sciences.

Mutiara, Restiani, dkk. 2015. *Uji Aktivitas Anti Oksidan Ekstrak Kulit Batang Kulit Kayu Manis (Cinnamonum burmanninessex BI.)dan Formulasinya dalam Bentuk Sediaan Masker Gel Peel Off*. Bandung.

Nagori B.P, Solanki R. 2011. *Role of Medicinal Plants in Wound Healing*. Journal of Medicinal Plant.

Wang.L. Weller C.L. 2006. *Recent Advances in Extraction of Nutraceuticals from Plants. Trends in Food Science and Technology*. 17.300-312

Zychowska M, Rojewska E, Przewlocka B, Mika J. 2013. *Mechanisms and pharmacology of diabetic neuropathy-experimental and clinical studies*, Institute of Pharmacology Polish Academy of Sciences