BIODIVERSITAS ISSN: 1412-033X
Volume 23, Number 8, August 2022 E-ISSN: 2085-4722
Pages: 4355-4360 DOI: 10.13057/biodiv/d230861

Antibacterial activity of Sunda porcupine quill extract (*Hystrix javanica*) against *Staphylococcus aureus*

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Manuscript received: 25 June 2022. Revision accepted: 9 August 2022.

Abstract. *Gifardi MD, Sutardi LN, Farida WR, Prawira AY, Agungpriyono S. 2022. Antibacterial activity of Sunda porcupine quill extract (*Hystrix javanica*) against* Staphylococcus aureus. *Biodiversitas 23: 4355-4360.* The Sunda porcupine (*Hystrix Javanica*) is a rare mammal with quill-like hair covering its body. Because the Sunda porcupine has extensive sebaceous glands, the neutral carbohydrate content is well-distributed and is known to have excellent wound healing. In addition, the high acid content of porcupine quills is believed to influence wound healing by inhibiting the growth of bacteria that cause infection on the skin. This study aims to demonstrate the antibacterial activity of the Sunda porcupine quill extract, determine the antibacterial ability of the Sunda porcupine quill extract, and examine the influence of concentration variations on the growth inhibition of *Staphylococcus aureus* bacteria. Well-diffusion testing of the antibacterial activity of the Sunda porcupine quill extract (so that it is well-diffusion). The test findings demonstrated that the Sunda porcupine quill extract possessed antibacterial properties. The antibacterial activity of porcupine quill extract was indicated by the diameter of the clear zone (inhibition zone) based on the variation of the extract concentration used, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%. The antibacterial activity of the Sunda porcupine quill extract was seen at concentrations of 100%, 50%, and 25%, with the average diameter of the clear zone being 14.88 mm ± 2.43; 12.24 mm ± 2.59; and 12.74 mm ± 3.69. The test results showed an effect of variations in the concentration of Sunda porcupine quill extract on the diameter of the clear zone ($P \leq \alpha = 0.05$) based on the one-way ANOVA test. Sunda porcupine quill extract with concentrations of 100%, 50%, and 25% is included in the category of strong antibacterial so that it can be used as an antibacterial agent.

Keywords: antibacterial, clear zone, extract, free fatty acid, porcupine quill.

INTRODUCTION

Porcupines are mammals that belong to the world's largest rodent group. In general, porcupines belong to one of two families: the Hystricidae family, also known as the old-world porcupine, and the Erethizsontidae family, also known as the new world porcupine (MacDonald 2006). The old-world porcupines are included in the infraorder piomorpha, which consists of 11 species (Rovie-Ryan et al., 2017). The new world porcupine belongs to the caviomorph rodent group piomorpha, which consists of 15 species (Voss et al. 2013). The old-world porcupines have long, flexible, and flat quills, while new-world porcupines have short spines with circular cross-sections and spikes at the ends (Tee et al., 2021). One of Indonesia's old-world porcupine species is the Sunda porcupine (*Hystrix javanica*). The Sunda porcupine inhabits natural woodland in several regions on the island of Java (Mustikasari et al., 2019). The Sunda porcupine is a rare mammal with cylindrical quills covering its upper body. Adult porcupines are covered with tens of thousands of spiky quills on their backs and tails (Olson and Lewis 1999). The keratin composition of porcupine quills is nearly identical to that of hair and nails. The quills of porcupines are modified hairs with sharpened tips. The largest quills often develop on the back, with the smallest on the face (Keating 2018).

Multiple community groups in Indonesia employ porcupine quills as a remedy for sickness. Porcupine quills are processed into flour and then used as medicine to treat acne by people in Kalimantan. The people of Central Java usually use porcupine quills as medicine for toothaches and ulcers (Inayah 2016). Porcupine quill ash is also used as a pain reliever for fractures by giving it orally (Kakati and Daulo 2002). To demonstrate the medicinal efficacy of porcupine quills, this belief must be substantiated by study findings. Several investigations have been conducted regarding the antibacterial activity of porcupine quills. The free fatty acid extract from the quills of the American porcupine (*Erethizon dorsatum*) is reported to possess antibacterial action against numerous gram-positive bacterial species (Roze et al. 1990). Prawira's (Prawira 2019) research on the Sunda porcupine revealed that the porcupine's skin contains fatty acids that have antibacterial properties. The fatty acid components from the porcupine's skin are present in the quills and have an antibacterial effect since they are obtained from the skin (Prawira et al., 2018). In addition, the sebaceous glands in porcupine quill follicles, which are oil glands, are reported to be significantly larger than hair oil glands (Prawira et al., 2019). The large size will release large amounts of oil into the porcupine quills so that oil and other lipids will be present on the surface of the quills and skin.

It was previously believed that the amount of free fatty acids in porcupine quills was connected to their antibacterial activity. Many studies have proven the effectiveness of free fatty acids from animal sources as antibacterial, for example, fatty acids extracted from donkey milk (Koutb *et al.* 2016), snakehead fish (*Channa striata*) (Rahman *et al.* 2018), and salmon (S*almo salar*) (Inguglia *et al.* 2020) is known to have antibacterial activity. Fatty acids contained in plants are also known to have antibacterial activity. Free fatty acid extracts from the *L. pumila* variety (Karimi et al. 2015) and coconut oil (Mena et al. 2020) inhibited the growth of gram-positive and gram-negative bacteria. 18.6% of the fatty acids in the quills of Sunda porcupines are free (Inayah et al., 2020). The high amount of free fatty acids in porcupine quills can be used as a guide for using porcupine quills as an antibacterial. Still, there isn't much information about how well they work or how antibacterial they are.

The skin, a body's barrier against infection with pathogenic microorganisms, normally harbors many normal flora microorganisms. Normal flora is found on the skin, such as *Staphylococcus spp*., corynobacteria, and fungi (Lacey et al. 2016). These microorganisms usually cause skin infections, one of which is *Staphylococcus aureus*. *S. aureus* causes infection by producing toxins, enterotoxins, and coagulases that damage cell membranes and induce cell death (Yokota et al., 2021). Wound infections are usually rare on the skin of the Sunda porcupine. The Sunda porcupine is known to have good wound healing even though the severity of the wound is quite high with minimal infection (Prawira 2019). This is an interesting finding to study. The lack of infection in the skin wounds of the porcupine is thought to be due to the fatty acid content in the spines. The effectiveness of porcupine quills against *S. aureus* bacteria as one of the bacteria that causes skin wound infections must be analyzed to prove these findings.

The purpose of this study was to examine the antibacterial activity of Sunda porcupine quill extract at different doses against *S. aureus* bacteria. Data on the antibacterial activity of the Javan porcupine spines are expected to be used in the biomedical field as an antibacterial alternative. This data is also expected to be information for veterinarians who work in captivity for Sunda porcupines. Given the evidence that porcupine quills have antibacterial activity, veterinarians do not need to give antibiotics too often to treat wounds in porcupines unless the wound condition requires special treatment to reduce contact with the porcupines.

MATERIALS AND METHODS

Research material

The porcupine quill samples were obtained from the Indonesian Institute of Sciences' Biological Research Center's Wildlife Captivity. Samples of quills were collected from quills that fall naturally from the porcupine's body. The collected quills are then properly cleansed to remove any dirt. The quills were placed in an airtight zipper bag and refrigerated until examination.

Work procedures

Number 002/KE.02/SK/6/2022 has been assigned by The National Research and Innovation Agency's Ethics Commission's Secretariat as ethical approval for this study.

Sunda porcupine quill extraction

The extraction and maceration procedure requires an oven, aluminum pan, scissors, blender, porcelain dish, analytical scale, glass container, 1L beaker glass, vacuum rotary evaporator, and 1.5ml microcentrifuge tube. In the meantime, the following components were utilized: Sunda porcupine quills, 3L n-hexane solvent, Whatman filter paper, and plastic wrap.

The tools used in the extraction and maceration process are oven, aluminum pan, scissors, blender, porcelain dish, analytical scale, glass container, 1L beaker glass, vacuum rotary evaporator, 1.5ml microcentrifuge tube. Meanwhile, the materials used were Sunda porcupine quills, 3L n-hexane solvent, Whatman filter paper, and plastic wrap.

The collected Sunda porcupine quills were then dried in an oven at 50ºC for 3 hours to reduce the moisture content. The porcupine quills are then cut into small pieces and ground into powder. The extraction of the quills of the Sunda porcupine is carried out by maceration. Extraction was carried out by weighing 100g of porcupine quill powder and then soaking it in n-hexane solvent in a ratio of 1:10 in a glass container for 3 x 24 hours. The extract was filtered using filter paper and then evaporated with a vacuum rotary evaporator at 37-40ºC to obtain a thick extract. Then the extract yield was calculated (Kemenkes RI 2017).

The calculation of the yield of the Sunda porcupine quill extract is carried out based on the formula below (Depkes RI 2000):

$$\% \text{ Yield} = \text{Extract Weight (g)Simplicia Weight (g)} \times 100\%$$

Test media creation

The test media consisted of a combination of Mueller Hinton Agar (MHA) media and a suspension of *Staphylococcus aureus*, which was isolated from milk in 2010 and is part of the collection of the Bacteriology Laboratory, Department of Animal Diseases and Public Health, IPB University. The test microorganisms were plated on nutrient agar and cultured at 35 degrees Celsius for 24 hours. The bacterial colonies that had grown on the media were then taken off with a needle loop and diluted with a sterile 0.9% NaCl solution until the turbidity met the standard of Mc. Farland (10^7 - 10^8CFU/ml) (Utomo *et al.* 2018). According to the packaging's formulation, 38g of MHA medium is utilized (2g beef extract; 17,5g casein hydrolysate; 1,5g starch; 17g aga*r*) and dissolved using 1 L of distilled water (the mixing process can be assisted by heating). The homogenized medium was sterilized for 20 minutes in an autoclave at 121°C. With a dropper, 1 ml of the test bacteria suspension was moved to a clean Petri dish. Then, 15 ml of MHA media that had already been made, mixed, and left to harden were added.

Antibacterial activity test of sunda porcupine quill extract

The testing of the antibacterial activity of the Sunda porcupine quill extract was of the experimental type. The research design was a completely randomized design (CRD) with seven treatment concentrations of porcupine quill extract and two replications per concentration. The well diffusion method was utilized to evaluate antibacterial activity against test microorganisms (Akerina et al., 2015). Wells with a diameter of 3 mm were drilled in MHA that had been combined with a suspension of S. aureus test bacteria using a sterile dropper. Extract with 100% concentration (200mg/200µL), 50% (100mg/200µL), 25% (50mg/200µL), 12,5% (25mg/200µL), 6,25% (12,5mg/200µL), 3,125% (6,25mg/200µL), 1,5625% (3,125mg/200µL) was made using dimethyl sulfoxide (DMSO) solvent then dripped into the well as much as 20 L. Positive control treatment using chloramphenicol antibiotic 30µg/disk, and negative control using n-hexane and 10% DMSO as solvent. The media was incubated at 37ºC for 24 hours. Antibacterial activity was indicated by forming a clear zone around the well and measured using a digital caliper.

Data analysis

For each treatment, the diameter of the clear zone was measured and analyzed using SPSS, a one-way ANOVA test, and Tukey's test with a significance threshold of 95% ($\alpha \leq 0{,}05$). The collected data is transformed into tables or graphs, which are subsequently explained in sentences, while concurrently, conclusions are drawn (Steel dan Torrie 1993).

RESULTS AND DISCUSSION

Antibacterial activity test

The test was conducted to demonstrate the antibacterial activity of the Sunda porcupine quill extract against the test microorganisms, as evidenced by creating a clean zone around the well. Figure 1 depicts the test findings of the inhibitory zone of the Sunda porcupine quill extract against *S. aureus* bacteria.

The antibacterial activity test of the Sunda porcupine quill extract showed positive results (a clear zone was formed) in the treatment with extract concentrations of 100% (1), 50% (2), 25% (3), and positive control chloramphenicol (C+). In comparison, the results were negative (no clear zone was formed) seen in the treatment with extract concentrations of 12.5% (4), 6.25% (5), 3.125% (6), and 1.5625% (7), as well as N-Hexane and DMSO solvents. The clear zone formed at treatment concentrations of 100%, 50%, and 25% can be used as initial evidence that the Sunda porcupine quill extract has antibacterial activity against *S. aureus* bacteria.

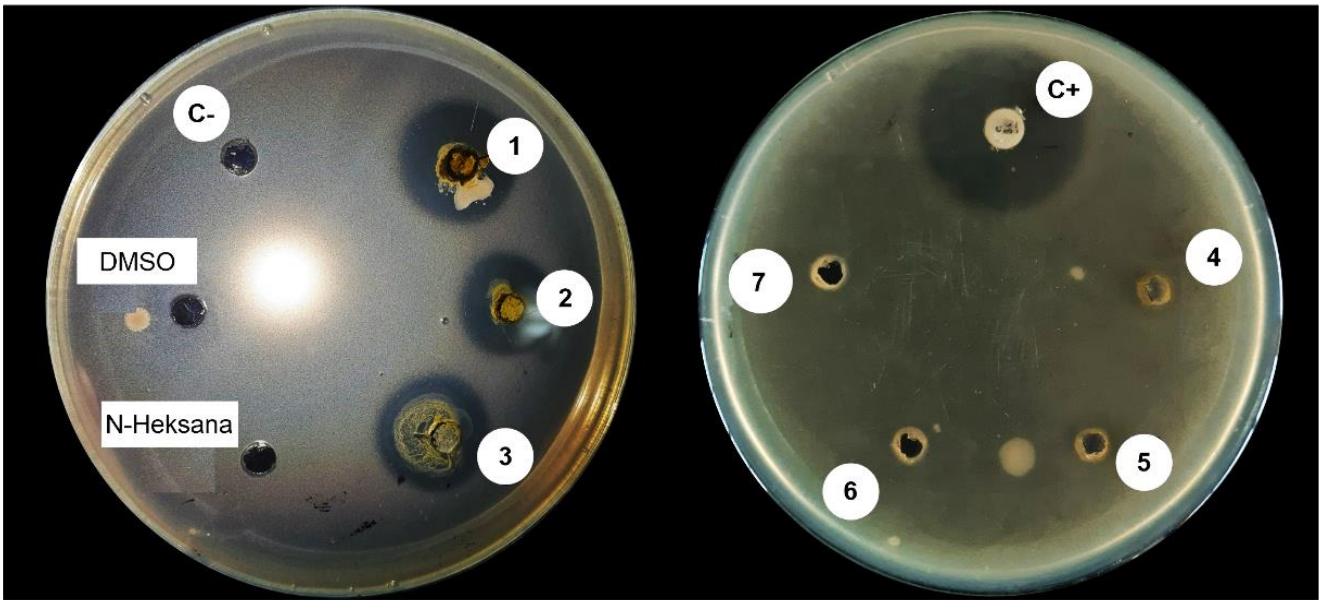

Figure 1. The results of the antibacterial activity of the extract of the Sunda porcupine quill extract against *S. aureus* bacteria using the agar well diffusion method. The negative control consisted of empty wells (C-), DMSO, and N-Hexane as solvents. The positive control was chloramphenicol (C+). Porcupine quill extract with successive concentrations: 100% (1), 50% (2), 25% (3), 12.5% (4), 6.25% (5), 3.125% (6), 1.56% (7).

Table 1. The results of the measurement of the average diameter of the clear zone of the Sunda porcupine quill extract against *S. aureus*

Treatment	Average clear zone diameter (mm) ± SD	Category
Porcupine quill extract		
100%	14.88±2.43[a]	Strong
50%	12.24±2.59[a]	Strong
25%	12.74±3.69[a]	Strong
12.5%	0±0[b]	No effect
6.25%	0±0[b]	No effect
3.125%	0±0[b]	No effect
1.5625%	0±0[b]	No effect
Chloramphenicol (+) 30 µg/disk	20.97±0.9[c]	Very strong

Note: Different superscripts (a, b, and c) in the same column showed significant differences (P<0.05) based on Tukey's test

Analysis of the effect of variations in the concentration of Sunda porcupine quill extract using the one-way ANOVA test

The extract of Sunda porcupine quill has demonstrated antibacterial action in creating a clean zone. The diameter of the clear zone for each treatment was then measured with a digital caliper for statistical analysis using one-way ANOVA and Tukey's test to determine the differences between treatments. Tukey's test is a commonly used method for detecting differences in pairwise comparisons with multiple repetitions to reduce standard errors and increase the probability of rejecting the null hypothesis. This test uses paired post-hoc testing to determine whether there is a difference between the mean of all pairs. This method tests every possible pair of all groups (Lee and Lee 2018). The objective of measuring the diameter of the clear zone is also to categorize the sensitivity of the Sunda porcupine quill extract based on its capacity to prevent bacterial development. The average diameter of the clear zone of the Sunda porcupine quill extract for each treatment is measured and shown in table 1.

Based on the results of the measurement of the diameter of the clear zone, the average diameter of the clear zone was the largest in the 100% extract concentration treatment. In comparison, the 12.5%, 6.25%, 3.125%, 1.5625% extract concentration treatments did not form a clear zone around the wells (Table 1). The results of the one-way ANOVA test showed that the concentration of porcupine quill extracts affected the diameter of the clear zone formed on the test medium with a significance value of 0.000 ($\leq\alpha$=0.05). Furthermore, further tests were carried out using Tukey's test to show differences between treatments to determine which treatments differed significantly from other treatments (Table 1). The test results show that the Sunda porcupine quill extract with a concentration of 100% has an insignificant difference in the diameter of the clear zone with an extract concentration of 50% and 25%, as well as a 50% extract concentration that is not significantly different from 25% so that these three treatments have the same antibacterial activity. Extract concentrations of 100%, 50%, and 25% had a significant difference in the diameter of the clear zone with treatment concentrations of 12.5%, 6.25%, 3.125%, and 1.5625%, so that the Sunda porcupine quill extract with a concentration of 25% was the lowest concentration which could inhibit the growth of *S. aureus* bacteria. The extract concentrations of 12.5%, 6.25%, 3.125%, and 1.5625% did not significantly differ between treatments based on the diameter of the clear zone, so these four treatments did not have sufficient ability to inhibit the growth of *S. aureus* bacteria. Treatment with concentrations of 100%, 50%, and 25% had a significant difference in the diameter of the clear zone compared to the positive control treatment with chloramphenicol which had the largest clear zone diameter (20.97mm ± 0.9). This shows that although it has antibacterial activity, the Sunda porcupine quill extract with concentrations of 100%, 50%, and 25% was not stronger than the chloramphenicol antibiotic in inhibiting the growth of *S. aureu*s bacteria.

Discussion

The maceration technique was chosen because of its simplicity (Zhang et al., 2018) and prevalence (Badaring et al., 2020). This technique can avoid harming thermolabile chemicals (Mukhriani 2014). N-Hexane was selected as the most effective solvent for the extraction of free fatty acids. It is known that porcupine quills have a high concentration of free fatty acids, which is believed to be related to the antibacterial properties of porcupine quills. This solvent's low boiling point facilitates the separation of the extract (Soetjipto et al., 2018). According to previous studies (Simplice et al., 2018), the extraction of oil from *Chrysicthys nigrodigitatus* fish using N-hexane as a solvent resulted in fatty acid content of 5.8 percent. A good solvent for fat extraction must-have features like a high affinity for fat, a low boiling point, and low toxicity. This solvent possesses all of these qualities (Mercer and Armenta 2011). This nonpolar solvent has a method of action that includes van der Waals interactions with long-chain hydrophobic fatty acids and neutral lipids, allowing it to dissolve fatty acids present in organic materials (Escorsim *et al.* 2018).

Most frequently, the agar well diffusion method is employed to test the antibacterial activity of a substance or material (Balouiri et al., 2016). This technique is typically applied to substances or chemicals that are soluble and capable of diffusing into the surrounding environment (Almoudi et al., 2018). Antibacterial activity was demonstrated by creating a clear zone on the test medium at 25 percent, 50 percent, and 100 percent concentrations of Sunda porcupine quill extract. The clear zone demonstrates a material's ability to suppress bacterial growth (Hau and Rohyati 2017). It is believed that the high content of free fatty acids contributes to the antibacterial action of the Sunda porcupine quill extract. Sunda porcupine quills are reported to contain 18.6% free fatty acids (Inayah et al., 2020). Sunda porcupine quills have the same percentage of free fatty acids as American porcupine quills (18.6 percent) (Roze et al. 1990).

Although in this study, no further tests were carried out to identify or separate the active compounds present in the Sunda porcupine quill extract, we expect that most of the components of the active ingredients that were successfully extracted were fatty acids. Extracts using n-hexane as a solvent, apart from being able to attract fatty acids, are also known to be able to attract phenolic and flavonoid compounds, but the levels are very small (Wijaya et al., 2017; Sari et al., 2021), so we expect the antibacterial activity of the extract of the Sunda porcupine quill extract derived from fatty acids. The estimation is based on using n-hexane as a solvent in the extraction process as it is known that n-hexane solution is known to be the best solvent for free fatty acid extraction. Extracts extracted using hexane contain a large diversity of fatty acids (Msimanga et al., 2013). n-hexane is the most widely used solvent for oil extraction, including fatty acids, because it easily dissolves oil (Kumar et al., 2017). Fatty acids are substances comprising long hydrocarbon chains and carboxylic acids. Fatty acids aid the body by regenerating the skin and enhancing the immune system and cell membrane function (Idris et al., 2020). Also recognized to have antibacterial effects against gram-positive and gram-negative bacteria are free fatty acids (Nguyen et al., 2017). Free fatty acids: the antibacterial mechanism of action of free fatty acids involves interfering with the electron transport chain process by binding to electron carriers, altering the integrity of the membrane and disrupting oxidative phosphorylation by lowering the membrane potential and proton gradient, inhibiting membrane enzymes such as glucosyltransferases, or causing bacterial cell lysis (Yoon et al. 2018). Free fatty acids offer a broad spectrum of antibacterial action, making them intriguing for use as antibacterial agents in medicine, agriculture, and food preservation, particularly in areas where antibiotics are restricted or even outlawed (Desbois and Smith 2010).

The effectiveness of porcupine quill extract at concentrations of 25%, 50%, and 100% against *S. aureus* bacteria was still inferior to that of chloramphenicol; nevertheless, based on the diameter of the clear zone, these three concentrations belonged to the strong category. Based on the diameter of the inhibition zone (clear zone), the ability of a compound to inhibit bacterial growth is classified into four categories: weak category with an inhibition zone of 5 mm or less, moderate with an inhibition zone of 5-10 mm, strong with an inhibition zone of 10-20 mm, and very strong with an inhibition zone of 20 mm or more (Davis and Scout 1971). The inadequacy of porcupine quill extract with a concentration below 25 percent to prevent bacterial growth was likely due to its insufficient fatty acid content. Several factors are known to influence antibacterial action, including the concentration of the extract, the number of metabolites, the diffusion strength of the extract, and the kind of bacteria inhibited (Jawetz et al. 1996). 25 percent is the minimal concentration of free fatty acids isolated from salmon heads (*Salmo salar*) that might suppress the growth of *S. aureus* bacteria, according to research (Inguglia et al., 2020). Although the fatty acid extract from the quills of the Sunda porcupine and the head of the Salmon has the same minimal concentration in inhibiting bacterial growth, additional research is still required to identify the metabolite compounds in the extract of the quills of the Sunda porcupine that are effective against S. aureus bacteria.

Staphylococcus aureus is a commensal bacterium found on the skin surface of the Sunda porcupine (Prawira et al., 2018); thus, the effectiveness of the Sunda porcupine quill extract is appropriate for inhibiting the growth rate of this bacterium. Chloramphenicol is recognized to exhibit potent antibacterial activity against the *S. aureus* bacterium (Pratiwi, 2008); hence, it is frequently utilized as a positive control in antibacterial activity tests, particularly those employing S. aureus bacteria. As a pathogen, *S. aureus* is very adaptive to drug resistance. This may restrict the therapeutic options available against *S. aureus*. This species can cause a broad spectrum of illnesses, ranging from minor skin infections to severe systemic disorders. Additionally, *S. aureus* is the cause of numerous infections associated with surgical incisions and catheter use. Due to this species' numerous virulence factors, it can inhabit many habitats with high infectious potential (Peacock *et al.* 2001).

ACKNOWLEDGEMENTS

The authors would like to thank the Institute for Research and Community Service, Bogor Agricultural University, for funding research in the Basic Research Program for Higher Education, grant no. 8/EI/KPT/2021.

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