Screening of Active Compounds and LC_{50} Toxicity Assay of Sunda Porcupine's (*Hystrix javanica* F. Cuvier 1823) Quills Crude Extract

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Abstract

Sunda porcupine (*Hystrix javanica* F. Cuvier 1823) is an endemic fauna from Indonesia that its quills are believed to have medical benefits by local people in some regions of Indonesia. However, the benefits have never been well recorded nor proven scientifically. Local people believe the Sunda porcupine's quills have efficacy to relieve property for treating toothache. There is limited research on Sunda porcupine's quills, especially the active compounds, which may affect toothache. This research aims to perform basic pharmacological experiments on Sunda porcupine's quill samples, which includes screening for the active compounds and determining the LC_{50} toxicity using brine shrimp lethality test (BSLT) method. Sunda porcupine's quills were first prepared into simplicia powder (60 mesh in size) and then extracted with 70% ethanol by maceration to produce crude extract. We found that the crude extract of Sunda porcupine's quills contains some active compounds, including alkaloids, flavonoids, saponins, triterpenoids, steroids, and peptides. The LC_{50} value of the crude extract was 2,683.19 ppm; thus, categorized as non-toxic. These findings can be used to identify the active compounds in Sunda porcupine's quills and can be used as a background for further research.

Keywords: *Hystrix javanica*, quills, crude extract, active compound, toxicity

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Introduction

The Sunda porcupine (*Hystrix javanica* F. Cuvier 1823) is a terrestrial rodent-mammal fauna native to Indonesia. It can be found in some regions of Indonesia, such as Java, Bali, Sumbawa, Flores, Lombok, Madura, and Tonahdjampea. The porcupine is active at night (nocturnal) and lives in groups. It is an abundant species and categorized as the least concern (LC) species based on IUCN Red List (van Weers, 1979; Van Weers, 1983; Woods & Kilpatrick, 2005; Aplin, 2016) (Figure 1).

The Sunda porcupine is known as an agricultural pest by the local people where the Sunda porcupine can be found (Farida, 2013). Local people hunt the porcupine to decrease the damage to crops since the porcupine eats various types of crops like tubers and corn. Some local people hunt the porcupine for its meat or medicine. They believe that the porcupine has medical benefits; however, this ethnomedicine is not well recorded. Anita *et al*. (2018) reported that the tail meat of Sunda porcupine has aphrodisiac potency. On the other hand, its family, the American porcupine quills (*Erethizon dorsatum*) has been reported to have antibiotic properties (Roze *et al*., 1990).

Exploration of active compounds from bioresources remains interesting. The research of active compounds from Sunda porcupine is still needs to be expanded despite local people believing much in its ethnomedicine. One of them is that the local people in some regions of Indonesia believe that Sunda porcupine's quills can be used for treating toothache. A basic pharmacological study should be performed to initiate more advanced research so that this ethnomedicine can be both well recorded and proven scientifically. This

research aims to perform basic pharmacological experiments to identify the active compounds in Sunda porcupine's quills, which includes screening of the active compounds and LC_{50} toxicity assay using brine shrimp lethality test (BSLT) method. The findings of this research could identify the active compounds in Sunda porcupine's quills and also can be used as background for further research.

Figure 1. Sunda porcupine (*Hystrix javanica* F. Cuvier 1823)

Materials and Methods

Simplicia Preparation. The Sunda porcupine's quills were the collection of Nutrition Laboratory, Zoology Division, Research Center for Biology, Indonesian Institute of Sciences taken from the remains of physiological research samples which has been approved by ethical commissions with the number B-15897/IPH/KS.02.04/XII/2019 signed by Deputy of Life Sciences, Indonesian Institute of Sciences. The fresh samples of quills were cleaned under running water to eliminate dirt and put under light at room temperature. The clean and dry sample was then weighed to obtain the wet weight. After that, the samples were dried in an oven at 50°C for 3 days to be simplicia. The simplicia was ready after the drying process and weighed to obtain the dry weight. The weight shrinkage percentage was calculated using the Formula 1.

$$\text{weight shrinkage (\%)} = \frac{\text{wet weight-dry weight}}{\text{wet weight}} \text{x100\% (1)}$$

The simplicia was mashed and sifted to obtain simplicia powder with the size of 60 mesh before being stored. The non specific character (water content) of simplicia powder was analyzed using AOAC method (2003) to ensure that the quality of simplicia powder was good enough before being stored and used for further purposes. The water content of simplicia should be less than 10% to minimize the risk of simplicia damage from enzymatic and microbial activities (Manoi, 2006). The amount of water inside the simplicia powder was eliminated by heating the simplicia powder at 105°C for 3 hours or until a constant final weight was reached. The weight before heating (Wo) and the weight after heating (Wt) were measured to determine the water content. The water content of the simplicia was calculated by the Formula 2.

$$\text{water content (\%)} = \frac{\text{Wo-Wt}}{\text{Wo}} \text{x100\% (2)}$$

The specific characteristics of simplicia powder were also determined based on organoleptic test. The test was performed to get the character of color, odor, taste, and texture of the simplicia powder.

Extraction. The obtained simplicia powder was extracted by maceration using 70% ethanol as the solvent. The ratio between the powder and the solvent was 1:10. Extraction was performed by shaking at 120 rpm for 3 days. The extraction process was repeated three times using the same simplicia powder. After the process, the filtrate was separated using filter paper. The filtrate was then dried to obtain crude extract. The drying process of filtrate was conducted using a rotary evaporator with temperature of 50°C at 120 rpm. The yield of crude extract was then calculated using the Formula 3.

$$\text{yield extract (\%)} = \frac{\text{weight of crude extract}}{\text{weight of simplicia powder}} \text{x100\% (3)}$$

The specific characteristics of the crude extract were also determined by an organoleptic test similar to that performed on simplicia powder. The characteristics tested were color, odor, taste, and texture of the crude extract. The characters can be used as identity of the extract, which distinguish it with other crude extract.

Screening of Active Compounds. The crude extract was screened qualitatively for the content of its active compounds. The qualitative analyses conducted in this research

were alkaloid, steroid-triterpenoid, flavonoid, saponin, tannin, and peptide assays.

Alkaloid assay. The assay was performed by the reagents of Meyer, Wagner, and Dragendorf. About 40 mg of crude extract was stirred firmly with ammonia 25%. Next, 20 mL of chloroform was added and crushed intently. The mixture was then filtered with filter paper to obtain the filtrate. The filtrate was mixed with 10% HCl while shaking to form several fractions. The top layer fraction solution was divided into three test tubes. Meyer's reagent was added to the first tube, drop by drop. A positive result is indicated by a white precipitate. Wagner's reagent was added to the second tube. A positive result is indicated by a brownish-red precipitate. Dragendorff's reagent is added to the third tube. A positive result is an orange-red brick precipitate (Aziz, 2015; Bintang, 2010; Harborne, 1996).

*Steroid-triterpenoid assay***.** The crude extract was macerated with 10 mL of ether for 2 hours in an evaporating dish and covered with aluminum foil. After that, it was filtered using filter paper and a separating funnel. Then the filtrate was taken and heated at 50°C until the residue was obtained. Then, sulfuric acid was added drop by drop to the residue until the green color was formed, indicating the presence of steroid, while the formation of red color indicates a positive triterpenoid (Balamurugan *et al*., 2019; Harborne, 1996).

Flavonoid assay. The crude extract was added with 100 mL of distilled water. Then, the mixture was heated for 5 minutes and filtered. The filtrate in the test tube was added with magnesium powder, concentrated HCl, and amyl alcohol. Next, the test tube was shaken quickly and was allowed to form several layers. A positive indicator of the presence of flavonoid compounds is the formation of orange color on the amyl alcohol layer (Nea *et al*., 2021).

Saponin assay. Flavonoid test result filtrates was added with 5 mL of 0.5 M alcoholic KOH in a tube reaction. The filtrates were shaken for 10 minutes and were allowed to stand for a few minutes. The formation of stable foam that does not disappear with the addition of one drop of 1% HCl in the test tube indicates the presence of a saponin group (Harbone,1996).

Tannin assay. The crude extract was added with 25 mL of distilled water, boiled for 15 minutes, cooled and filtered. The filtration results were divided into two parts. The first filtrate was added with 1% iron (III) chloride solution drop-wise until a dark blue or blackish green color formed as a positive indicator of the tannin group. The second filtrate was added with Stiasny's reagent and heated in a water bath. The formation of a pink precipitate indicated condensed tannins. Next, the pink precipitate was filtered using filter paper and a separating funnel, then the filtrate was saturated with sodium acetate, and a few drops of 1% $FeCl_3$ were added. The dark blue color indicates the presence of false-positive tannins (Benzidia *et al*., 2019; Djamil & Anelia. T, 2009).

Peptide assay. The crude extract was reacted using Bradford's reagent. The positive reaction is signed by the bluish-purple solution at the end of the reaction (Bradford, 1976; Bintang, 2010).

Toxicity of LC_{50}. The toxicity was assayed using the method of brine shrimp lethality test (BSLT). The shrimp *Artemia salina* was used for the assay. The shrimp eggs were put into an aquarium containing seawater and aerated for 48 hours under light at room temperature. After 48 hours, the eggs will hatch into nauplii. A series of solutions of the Sunda porcupine's quills crude extract was prepared in a concentration of 1,500 pp;, 1,000 ppm; 500 ppm; 250 ppm; 125 ppm; 62.5 ppm; 31.25 ppm; and 0 ppm (placebo). Each concentration was placed on a vial test and was performed triplo (Jelita *et al*., 2020; Meyer *et al*., 1982; Zuraida, 2018). The assay was conducted by placing 10 nauplii in each vial of concentration. After 24 hours, the nauplii which were still alive were counted and the percentage of mortality was calculated using the Formula 4.

$$\text{Mortality (\%)} = \frac{\text{number of dead nauplii}}{\text{number of tested nauplii}} \text{x}100 \quad (4)$$

The percentage of mortality was then analyzed by probit using SPSS 25. The value of LC_{50} was then determined.

Results

Organoleptic Characteristics of Simplicia Powder and Crude Extract.

The porcupine's quills were prepared into small cuts of simplicia, simplicia powder, and crude extract (Figure 2). Both the simplicia powder and the crude extract were analyzed through an organoleptic test and the results are listed in Table 1. The colors of simplicia powder and crude extract were totally different. The color of simplisia powder was gainsboro gray into light gray while the crude extract was golden yellow. The characteristics of simplicia powder were relatively weak (had no taste and odor), while the characteristics of the crude extract were strong (had specific odor and taste). The weak characters of the simplicia powder occurred because the compounds that gave the characters did not concentrate, while the compounds in the crude extract were well concentrated.

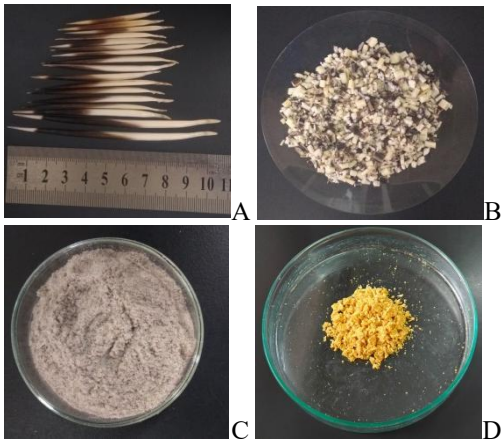

Figure 2. A. Sunda porcupine's quills; B. small cuts of simplicia of Sunda porcupine's quills; C. simplicia powder of Sunda porcupine's quills; D. crude extract of Sunda porcupine's quills.

The Shrinkage of Simplicia Preparation and The Yield of Crude Extract.

The Sunda porcupine's quills were well prepared as simplicia powder and crude extract. The parameters we used in this study were the percentage of weight shrinkage, the water content of simplicia, and the percentage of yield extract. The results of these parameters are shown in Table 2. The weight shrinkage of the quills was relatively lower than samples from plant resources. As a consequence, the yield extract of the quills was also relatively lower than plant resources. The water content was less than 10%, indicating that the drying process has been well performed and the quality of the simplicia produced was good for both to be saved and extracted.

Table 1. Results of organoleptic test on simplicia powder and crude extract of Sunda porcupine's quills

Organoleptic Characters	Simplicia Powder	Crude Extract
Color	gainsboro gray into ligh tgray	Golden yellow
Odor	odorless, bit musty, bit dusty	pungent, acidic, unpleasant smell
Taste	tasteless, light dusty	salty and bitter
Texture	lightweight, easy to fly, smooth bit rough	sticky, paste

Table 2. Extraction parameters of Sunda porcupine's quills

Parameters	Result
Weight shrinkage (%)	12.32 ±1.51
The water content of simplicia (%)	9.1 ± 0.13
Yield extract (%)	3.32%

Screening of Active Compound.

The screening results of active compounds of the crude extract of Sunda porcupine's quills is shown in Table 3. The crude extract of Sunda porcupine's quills gave a positive result for several groups of active compounds, including alkaloid, flavonoid, saponin, steroid/ triterpenoid, and peptide. The crude extract did not give a positive result on tannin assay as it did not form dark blue solution after the reaction.

Toxicity of LC_{50}.

The toxicity test was performed using the brine shrimp lethality test (BSLT), which used *Artemia salina* as an animal model. The extract is considered as non-cytotoxic if LC_{50} > 1,000 ppm, low cytotoxicity if LC_{50} between 500 and 1,000 ppm, moderate cytotoxicity if LC_{50} between 100 and 500 ppm, and high cytotoxicity if LC_{50} < 100 ppm (Meyer, 1982). The result is presented in Table 4. The data showed that the percentage of mortality increased with the increasing concentration of the extract. The extract exposured at 500 ppm and 1,000 ppm has the same mortality percentage. The extract would exert the same effect between 500 ppm and 1,000 ppm, may be due to the concentration of 500 ppm being the lower limit and 1,000 ppm being the upper limit, which could give effect 26.67%

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mortality. The crude extract of Sunda porcupine's quills is categorized as non-toxic since the value of LC_{50} is 2,683.19 ppm, which is higher than 1,000 ppm.

Table 3. Screening results of active compounds in the Sunda porcupine's quills

Group of Active Compound	Type of Assay	Observations	Result
Alkaloid	Meyer	white precipitate	+
	Wagner	brown precipitate	
	Dragendorf	orange precipitate	
Flavonoid	Bate Smith & Metcalf	pale-transparent orange	+
Saponin	Froth	stable foam	+
Steroid/ triterpenoid	Lieberman-Burchard/ Keller Killiani	red (initial-center)/ green (late-side)	+
Tannin	Stiasny		
$FeCl_3$	orange precipitate (doesn't form dark blue)	-	
Peptide	Brad-ford	dark blue	+

Table 4. The toxicity of LC_{50}

Concentration (ppm)	Mortality (%)	LC50 (ppm)	Toxic Category
0	0	2,683.19	non-toxic
31.25	0		
62.50	0		
125.00	6.67		
250.00	10.00		
500.00	26.67		
1,000.00	26.67		
1,500.00	33.33		

Discussion

Simplicia preparation of Sunda porcupine's quills is the first procedure performed before extracting the active compounds of the quills. The fresh quills must be cleaned up from any dirt to minimize any contaminants that might be carried along. The drying process was performed by using an oven at temperature 50°C, which aimed to minimize the risk of damage to the active compounds. The percentage of shrinkage of Sunda porcupine's quills was about 12.32%. It was much lower compared to plant resources. The water content of Sunda porcupine's quills was 10% as reported by Inayah *et al*. (2020), while the water content of plant resources like vegetables and fruits is about 80-95% (Khan *et al*., 2017). Beside that, Inayah *et al.* (2020) reported that the major component of Sunda porcupine's quills was protein that reaches more than 90%, different to other tissue from bioresources that the major component is water, fills up the cell and the extracellular environment.

The organoleptic characters of simplicia powder were gainsboro gray into light gray in color, odorless-bit, musty-bit, dusty odor, tasteless-light, dusty taste, and lightweight-smooth bit with a rough texture. Meanwhile, the crude extract characters was golden yellow in color, had pungent-acidic-unpleasant smell, salty-bitter taste, and sticky like paste texture.

The structure of the porcupine's quills was hard and sharp, so we needed to prepare it into small pieces before mashing them into powder. The hardness of the quills may be caused by the high protein content, which is thought to be keratin protein (Inayah *et al*., 2020). The small cuts of the dry quills were then called simplicia and then mashed into powder. The simplicia powder had a water content of about 9.1%, less than 10%, which indicated that the simplicia is ready to be extracted or stored since the low water content of simplicia inhibits microbial growth.

The extraction was conducted using the maceration technique with 70% ethanol. The yield of the crude extract was about 3.32%. Compared to plant resources, the crude extract of porcupine's quills was much lower than that obtained from plant resources, which is up to 10-30% (Dinata *et al*., 2015; Rahman *et al*., 2017; Wirnawati *et al*., 2020). It may be caused by the difference in the major the components that fill up the tissue matrix since the Sunda porcupine's quills are filled with protein while plant resources are filled up with water. The protein, which acts as the major component in the Sunda porcupine's quills, cannot be removed from the quills by the drying process and still contributes to the weight of the simplicia during the extraction process. In contrast, water, a major component in plant resources, can be removed in high quantities during the drying process. Consequently, the active compounds inside the simplicia of plant resources are more

concentrated than the simplicia of Sunda porcupine's quills.

The number of active compounds from animals found in various sources is predicted about 50,000-100,000. The group of active compounds in animals generally functions as antibiotic (Berdy, 2005). The qualitative test found that the Sunda porcupine's quills contain alkaloids, flavonoids, saponins, triterpenoids, and peptides. These compounds may affect human health and interact with various receptors to induce metabolic effects.

Alkaloids are nitrogen compounds with unique molecular structures found in nature (Srivastava & Singh, 2020). The types of alkaloids include caffeine, morphine, codeine, reserpine, and so on. One of the functions of alkaloids, especially in animals, is as a defense system against pathogens. Therefore, alkaloids are potential as antibacterial, antioxidant, and anti-inflammatory (Atanasov *et al*., 2015; Azam *et al*., 2003). Based on the results of this study, the Sunda porcupine's quills contain alkaloids that may have the potential as antibacterial, antioxidant, or anti-inflammatory properties.

Flavonoids are phenolic compounds known as natural products. The source of flavonoids in animals is mainly from plant-based diet, and a small part is biosynthesized *in situ* (Kumar & Pandey, 2013; Yao *et al*., 2004). Many studies found that flavonoid compounds act as an anti-inflammatory in several disease cases, e.g., pulpitis. Flavonoids play a role in suppressing several cytokine pro-inflammation and endogenous enzymes that induce an inflammation response, such as cyclooxygenase (COX) (Choy *et al*., 2019; Maleki *et al*., 2019). The crude extract of Sunda porcupine's quills contains flavonoids and may be potential as anti-inflammation.

Saponins are organic compounds primarily found in plants and a small part in animals (Podolak *et al*., 2010). Types of saponins include triterpenoid saponin, steroidal saponin, and saponin alkaloids in the presence of sugar bonds (Ashour *et al*., 2019). One of the functions of saponins is antibacterial with various mechanism pathways. This study showed that Sunda porcupine's quills contain saponins that may cause cell lysis in bacteria by damaging the membrane permeability. Not only saponins but also steroids/triterpenoids compounds were found in Sunda porcupine's quills. Positive identification of steroids/triterpenoids was shown by the formation of red color (initial center) using Lieberman-Burchard method and green color (late-side) using the Keller Killian method. In general, the biological activity of steroids/triterpenoids as analgesics is relieving pain due to inflammation (Del Grossi Moura *et al*., 2018; Howes, 2018). It is worth further discovering the function of active compounds in Sunda porcupine's quills for treating toothache with more attractive methods.

Peptides are commonly the primary metabolites since peptides can be found as structural or functional components inside living organisms. Recent studies showed that peptides can perform as secondary metabolites since they had antimicrobial activity, hence, are grouped as antimicrobial peptides (AMPs). The AMPs are recently known as host defense peptides that can be found in all forms of life. The molecules of AMPs are commonly short and negatively charged. The AMPs may have the potential to be antimicrobial, antiviral, antifungal, immunomodulator, and wound healer (Kumar *et al*., 2018; Mahlapuu *et al*., 2016). The AMPs can be found in the outer part of vertebrate body in the skin case of the frog *Xenopus laevis* containing AMPs of margainins (Zasloff, 1987). The quills are the outer part of Sunda porcupine's body and act as a defense system. The qualitative result showed that the crude extract of Sunda porcupine's quills was positive for peptides based on Bradford reaction. The peptides inside the crude extract may be AMPs; therefore, may have potential function like AMPs.

The crude extract of Sunda porcupine's quills was categorized as non-toxic since the value of LC_{50} is more than 1,000 ppm. We used brine shrimp (*Artemia salina* Leach) since they are sensitive to toxic compounds and are easier to handle than mice (Awaludin *et al*., 2020). The result showed that the crude extract of Sunda porcupine's quills had a high LC_{50} value (>1,000 ppm), and low mortality (<50%) indicated that the extract was non-toxic. Other studies had different results from herbs or botanicals with significant toxic potential and high bioactivity (Charen & Harbord, 2020). Toxicity is directly proportional to bioactivity. Therefore, the crude extract of Sunda porcupine's quills has low bioactivity against brine shrimp, but has not been tested on other types of organisms

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such as bacteria, fungi, or other pathogens. Further investigation using other animal models to find out the potential and characteristics of pharmacology issues of Sunda porcupine's quills is needed.

We have shown that the crude extract of Sunda porcupine's quills contains active compounds of alkaloids, flavonoids, saponins, steroids, triterpenoids, and peptides group. These results suggest that the extract may have potential as antibacterial, antiviral, antioxidant, antifungal, immunomodulator, wound healer, anti-inflammatory, and analgesics. As reported by Roze *et al*. (1990), the quills of New World porcupines are coated in fatty acids from exocrine secretions that prevent bacterial growth and are likely to prevent infection from self-inflicted wounds. However, the potentials need deeper investigation through further research using *in vitro*, *in vivo*, and *in silico* approaches.

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References

Anita, S., Agusta, A., Farida, W. R., Nugroho, H. A., & Wulansari, D. (2018). A preliminary study of aphrodisiac property from porcupine tail meat ethanol extract in male mice. *Zoo Indonesia*, *26*(1).

AOAC. (2003). Official Methods of analysis. Vol.I. 17th ed. Association of Analytical Washington, DC, USA.

Aplin, K. (2016). *Hystrix javanica*. The IUCN Red List of Threatened Species.

Ashour, A. S., El Aziz, M. M. A., & Gomha Melad, A. S. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal of Nanomedicine Research*, *7*(4). https://doi.org/10.15406/jnmr.2019.07.00199.

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, *33*(8), 1582-1614. https://doi.org/10.1016/j.biotechadv.2015.08.001.

Awaludin, Kartina, Maulianawati, D., Manalu, W., Andriyanto, Septiana, R., Arfandi, A., & Lalang, Y. (2020). Phytochemical screening and toxicity of ethanol extract of *Sauropus androgynus*. *Biodiversitas*, *21*(7). https://doi.org/10.13057/biodiv/d210712.

Azam, S., Hadi, N., Khan, N. U., & Hadi, S. M. (2003). Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. *Medical Science Monitor*, *9*(9).

Aziz, M. A. (2015). Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits. *Journal of Integrative Medicine*, *13*(3). https://doi.org/10.1016/S2095-4964(15)60179-0.

Balamurugan, V., Fatima, S., & Velurajan, S. (2019). A guide to phytochemical analysis. *International Journal of Advance Research and Innovative Ideas in Education*, *5*(1), 236-45.

Benzidia, B., Barbouchi, M., Hammouch, H., Belahbib, N., Zouarhi, M., Erramli, H., Ait Daoud, N., Badrane, N., & Hajjaji, N. (2019). Chemical composition and antioxidant activity of tannins extract from green rind of *Aloe vera* (L.) Burm. F. *Journal of King Saud University - Science*, *31*(4), 1175-1181. https://doi.org/https://doi.org/10.1016/j.jksus.2018.05.022.

Berdy, J. (2005). Bioactive microbial metabolites. *The Journal of Antibiotics*, *58*(1), 1-26.

Bintang, M. (2010). *Biokimia: Teknik Penelitian*. Penerbit Erlangga.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254.

Choy, K. W., Murugan, D., Leong, X. F., Abas, R., Alias, A., & Mustafa, M. R. (2019). Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NFκB) signaling in cardiovascular diseases: A mini review. *Frontiers in Pharmacology*, 10,1295. https://doi.org/10.3389/fphar.2019.01295.

Charen, E., & Harbord, N. (2020). Toxicity of herbs, vitamins, and supplements. *Advances in Chronic Kidney Disease*, *27*(1), 67-71. https://doi.org/10.1053/j.ackd.2019.08.003.

Del Grossi Moura, M., Lopes, L. C., Silva, M. T., Barberato-Filho, S., Motta, R. H. L., & De Cássia Bergamaschi, C. (2018). Use of steroid

and nonsteroidal anti-inflammatories in the treatment of rheumatoid arthritis: Systematic review protocol. *PloS One*, *16*(4), e0248866. https://doi.org/10.1097/MD.0000000000012658.

Dinata, D. I., Supriyadi, D., Djafar, G., Syerliana, S., Wijayanti, W., & Suherman, S. E. (2015). Effect of adding granul basil (*Ocimum americanum*) as antioxidants in fried foods. *Indonesian Journal of Pharmaceutical Science and Technology*, *2*(1). https://doi.org/10.15416/ijpst.v2i1.7807.

Djamil, R., & Anelia. T. (2009). Penapisan Fitokimia Uji BSLT dan uji antioksidan ekstrak metanol beberapa spesies papilionaceae. *Jurnal Ilmu Kefarmasian Indonesia*, *7*(2).

Farida, W. R. (2013). The physical and chemical characteristics of Sunda porcupine meat (*Hystrix javanica* F. Cuvier, 1823) given additional concentrate feed. *Jurnal Biologi Indonesia*, *9*(2), 311-325.

Fardiansyah, (2020). *Mengenal lebih dekat landak jawa, durinya bisa obati sakit gigi*. [Online]. Retrieved from https://techno.okezone.com/read/2020/06/11/56/2228231/mengenal-lebih-dekat-landak-jawa-durinya-bisa-obati-sakit-gigi.

Harborne, J. B (1996). *Metode Fitokimia : Penuntun Cara Modern Menganalisa Tumbuhan*. Bandung, ITB Press.

Howes, M. J. R. (2018). Phytochemicals as anti-inflammatory nutraceuticals and phytopharmaceuticals. *Immunity and Inflammation in Health and Disease* (pp. 363-388). Academic Press.

Inayah, N., Farida, W. R., & Purwaningsih, E. (2020). Microstructure of quills in Sunda porcupine *Hystrix javanica* (F. Cuvier, 1823). *Jurnal Biologi Indonesia*, *16*(1), 81-88. https://doi.org/10.47349/jbi/16012020/81

Jelita, S. F., Setyowati, G. W., & Ferdinand, M. (2020). Uji toksisitas infusa *Acalypha Siamensis* dengan metode Brine Shrimp Lethality Test (BSLT). *Farmaka*, *18*(1), 14-22.

Khan, M. I. H., Kumar, C., Joardder, M. U. H., & Karim, M. A. (2017). Determination of appropriate effective diffusivity for different food materials. *Drying Technology*, *35*(3), 335-346.

Kumar, P., Kizhakkedathu, J. N., & Straus, S. K. (2018). Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules*, *8*(1), 4.
https://doi.org/10.3390/biom8010004

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An Overview. *The Scientific World Journal*, *2013*, 162750. https://doi.org/10.1155/2013/162750

Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: An emerging category of therapeutic agents. *Frontiers in Cellular and Infection Microbiology*, *6*, 194. https://doi.org/10.3389/fcimb.2016.00194.

Maleki, S. J., Crespo, J. F., & Cabanillas, B. (2019). Anti-inflammatory effects of flavonoids. *Food Chemistry*, *299*, 125124.

Manoi, F. (2006). Pengaruh cara pengeringan terhadap mutu simplisia sambiloto. *Buletin Penelitian Tanaman Rempah dan Obat, 17*(1), 1-5.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. j, & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, *45*(05), 31-34.

Nea, F., Bitchi, M. B., Genva, M., Ledoux, A., Tchinda, A. T., Damblon, C., Frederich, M., Tonzibo, Z. F., & Fauconnier, M. L. (2021). Phytochemical investigation and biological activities of *Lantana rhodesiensis*. *Molecules*, *26*(4).

Podolak, I., Galanty, A., & Sobolewska, D. (2010). Saponins as cytotoxic agents: A review. *Phytochemistry reviews : Proceedings of the Phytochemical Society of Europe*, *9*(3), 425-474.

Rahman, S. M., Shohael, A. M., Ahamed, T., & Rahman, S. K. M. (2017). Thin layer chromatographic profiling and phytochemical screening of six medicinal plants in Bangladesh. *International Journal of Biosciences (IJB)*, *11*(1), 131-140. https://doi.org/10.12692/ijb/11.1.131-140.

Roze, U., Locke, D. C., & Vatakis, N. (1990). Antibiotic properties of porcupine quills. *Journal of Chemical Ecology*, *16*(3), 725-734. https://doi.org/10.1007/BF01016483.

Srivastava, A., & Singh, V. (2020). Physiological role of some alkaloids*, Journal of Medicine and Surgical Sciences*, *2*(3).

Van Weers, D. J. (1979). Notes on Southeast Asian Porcupines (Hystricidae, Rodentia). IV. On the taxonomy of the subgenus Acanthion F. Cuvier, 1823 with notes on the other taxa of the family. *Beaufortia*, *29*(356), 215-272.

Van Weers, D. J. (1983). Specific distinction in Old World porcupines. *Der Zoologische Garten, Jena*, *53*, 226-232.

Wirnawati, W., Mentari, I., & Pernando, N. (2020). The characterization of simplicia and extract of clove leaves (*Syzigium aromaticum* Merr. Et Perry L) for herbal mouthwash active ingredients. *Jurnal Ilmu Kesehatan*, *8*, 26-33. https://doi.org/10.30650/jik.v8i1.1377.

Woods, & Kilpatrick. (2005). Infraorder Hystricognathi Brandt, 1855. *Mammal Species of the World. A Taxonomic and Geographic Reference*, *2*, 1538-1600.

Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R., & Chen, S. S.

(2004). Flavonoids in food and their health benefits. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *59*(3), 113-122. https://doi.org/10.1007/s11130-004-0049-7.

Zasloff M. (1987). Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proceedings of the National Academy of Sciences of the United States of America*, *84*(15), 5449-5453. https://doi.org/10.1073/pnas.84.15.5449.

Zuraida, Z. (2018). Analisis toksisitas beberapa tumbuhan hutan dengan metode Brine Shrimp Lethality Test (BSLT). *Jurnal Penelitian Hasil Hutan*, *36*(3). https://doi.org/10.20886/jphh.2018.36.3.239-246