Media Farmasi: Jurnal Ilmu Farmasi (Journal of Pharmaceutical Science) e-ISSN 2503-5223
Vol. 20, No. 1, March 2023, pp. 1-7
http://journal.uad.ac.id/index.php/Media-Farmasi/index

Antibacterial Activity of Sappan (*Caesalpinia sappan* L.) Wood Methanol Extract Against *Staphylococcus epidermidis*

Waro Atunnisa, Irfan Zamzani*, Siti Nashihah

Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, Barito Kuala, Kalimantan Selatan 70581, Indonesia
*corresponding author: e-mail: irfan.zamzani@umbjm.ac.id

ARTICLE INFO

Article history
Received: 08-10-2021
Revised: 18-04-2022
Accepted: 02-09-2022

Keywords
Caesalpinia sappan L.
Antibacterial
S. epidermidis
Ultrasound-Assisted Extraction (UAE)

ABSTRACT

The entry and proliferation of microorganisms, such as bacteria, cause skin infections. One of the bacteria that causes skin infections is *Staphylococcus epidermidis*. Sappan (*Caesalpinia sappan* L.) wood has been known to have various pharmacology activities, one of which is antibacterial, so its activities need to be developed and improved. This study aimed to determine the activity of the methanol extract of Sappan (*C. sappan* L.) wood as an antibacterial against S. epidermidis. This research was conducted by extracting Sappan wood powder with 96% methanol as solvent using the Ultrasound-Assisted Extraction (UAE) method and testing for antibacterial activity with concentrations of 25%, 50%, 75% and 100% using the disc method. Results showed that the methanol extract of Sappan wood had an antibacterial activity; the highest inhibition zone at a concentration of 75% was 29.25 mm. Sappan wood was expected to be a promising alternative therapy for overcoming acne problems and can increase economic value for the wider community.

1. Introduction

Infectious diseases are caused by bacteria, one of which is skin infections. The presence of touch stimulation, pain or bad influence from outside the body will generally interfere with the skin. These disorders can cause the skin to be exposed to the disease. Boils, acne and eczema are diseases caused by bacteria (Retnaningsih et al., 2019). *Staphylococcus epidermidis* is a normal microbiota often found on humans' skin and mucous membranes. S. epidermidis is a bacterium consequent to diseases that spread throughout the body, especially the skin surface, which is its natural habitat. Infections caused by these bacteria generally have typical signs such as abscess formation. Bacteria can cause skin infections, ulcers, wounds, and inflammatory infections accompanied by pain during the process of abscess formation, so treatment is needed to remove the fluid and restrict the growth and spread of bacteria (Rosidah et al., 2018).

Sappan is a woody plant often used for its stem (Dharmayanti & Arjita, 2019). Sappan has been found in Indonesia and is included in the Fabaceae family. Empirically, it is known to have many benefits for treatment and is often consumed as a health drink by people. Sappan is known as traditional medicine with many benefits such as antimicrobial treatment, impure blood, antioxidants, allergies, boosting immunity, and anti-inflammatory and diabetes treatment (Cahyaningtyas et al., 2019).

In a previous study by Retnaningsih et al. (2019), a 0.1 mg/disc concentration was able to provide antibacterial activity against *S. epidermidis*. Cahyaningtyas et al. (2019) reported that ethanol extract of Sappan wood also showed antibacterial activity against *S. aureus* by dilution and diffusion methods. owever, so far, there has been no research on the antibacterial activity test of Sappan wood against *S. epidermidis* bacteria. It is necessary to test the activity of Sappan wood methanol extract against *S. epidermidis* bacteria using the *Ultrasound-Assisted Extraction* (UAE) method.

2. Materials and Methods

Equipment used in this research were a 40 mesh sieve, analytical balance, blender (Panasonic), water bath sonicator (Biobase), laminar airflow (LAF) (robust), aluminium foil, incubator (memmert), autoclave (tech mech), 10 cm diameter petri dish (Herma), test tube (Iwaki), 250 and 500 mL Erlenmeyer (pyrex), 100 mL measuring flask (pyrex), calliper, round loop, 5 mL syringe, tweezers, porcelain cup, 50 mL and 250 mL beaker glass (pyrex) scissors, label paper.

Materials used were Sappan wood (*C. sappan* L.) taken from Bangshadow Village, Bantarkawung District, Brebes Regency, 96% methanol solvent, aqua dest, nutrient agar (NA) media, dimethyl sulfoxide (DMSO), pure culture of S. epidermidis bacteria ATCC 12228 purchased from Yogyakarta health laboratory service, two µg clindamycin disc paper, blank disc paper, NaCl 0.9%, 2N HCl, concentrated HCl, H_2SO_4, $BaCl_2.2H_2O$, Dragendorf reagent, Mayer reagent, $FeCl_3$, Mg tape, gelatin salt, chloroform, anhydrous acetic acid, cotton and filter paper.

2.1. Sappan wood simplicial preparation

Samples of sappan wood (Caesalpinia sappan L.) were obtained from Bangshadow Village, Bantarkawung District, Brebes Regency, Central Java. Samples were taken in the form of wood cores from the sappan tree. Wet sorting was done by removing twigs, branches and bark of sappan, then washing and shaving using electric wood shavings. 2.2 kg of wet sappan wood shavings were weighed, dried by sunlight heating, and covered with a black cloth after the simplicia dried. They were crushed in the blender until they became powder and weighed, then placed in a container.

2.2. Sample extraction by UAE method

Sappan sawdust was sieved through a 40-mesh sieve. A total of 50 grams of sawdust was dissolved with 500 ml of 96% methanol solvent (1:10) and then placed in Erlenmeyer. The sample was extracted for 30 minutes at a temperature of 40˚C. This extraction was carried out eight times so that the total comparison of the sample used was 400 grams, and the solvent was 4000 mL. The mixture of solvents and solids was separated using a funnel and filter paper, and then the extract solution was concentrated by leaving it at room temperature until it thickened (Neswati & Ismanto, 2018).

2.3. Phytochemical Screening

Phytochemical screening was carried out to detect alkaloids, flavonoid tannins and polyphenols, steroids and triterpenoids and saponins in sappan wood extract solution using the colour method (Al Kadri et al., 2019).

2.4. Preparation of test bacteria

2.4.1. Rejuvenation of test bacteria culture

The test bacteria in the form of S. epidermidis derived from pure cultures took one inoculating loop, inoculated on oblique nutrient agar media, scratched, and then incubated at 37˚C for 24 hours (Wijayati et al., 2014).

2.4.2. Preparation of McFarland's solution

This solution was prepared by mixing 99.5 mL of 0.36 N H2SO4 with 0.5 mL of $BaCl_2.2H_2O$ solution in Erlenmeyer. Then shake until a cloudy solution was formed. The standard for the turbidity of bacterial suspension was 1.5 x 108 CFU/mL.

2.4.3. Bacterial suspension preparation

Preparation of test bacterial suspension by taking the results of S. epidermidis bacterial rejuvenation and then dissolving it in (0.9% NaCl) 4 mL of physiological salt aseptically. The level of bacterial turbidity was compared with the McFarland standard (Arista et al., 2013).

2.4.4 Preparation of Nutrient agar (NA) media

Media was prepared by dissolving 4.2 grams of NA into 150 mL of distilled water (28 g/1000 mL) and then heated and stirred in an Erlenmeyer flask using a water bath until it boiled and was homogeneous. The mouth of the Erlenmeyer flask was plugged using cotton, covered with aluminium foil, and then sterilized using an autoclave at 121˚C and 1.5 atm pressure for 15 minutes. After sterilization, 15 mL of NA was put into a petri dish which would be used in an antibacterial test (Suryana et al., 2017).

2.4.5. Extract concentration manufacture

Sappan wood methanol extract was then made in several concentrations, namely 25%, 50%, 75%, and 100% w/v. For a concentration of 25%, 1.25 g of sappan wood methanol extract was weighed and then dissolved in 5 mL of solvent (DMSO 2%). At other concentrations, the same steps were carried out.

2.4.6. Negative and positive control

The positive control used as a comparison antibiotic was clindamycin, an antibiotic often chosen in acne treatment and suitable for inhibiting S.epidermidis bacteria, while the negative control used was 2% DMSO, a substance used to dilute the test compound. The aim was to antibacterial test results of the compound that would not be affected by the solvent used as the diluent.

2.4.7. Sappan Wood Extract Antibacterial Testing

A sterile cotton swab was dipped in a bacterial suspension of S.epidermidis, measured with McFarland standard, and then scratched evenly on the surface of the NA medium. Each paper disc was soaked in methanol extract of sappan wood for 1 hour with the concentration of 25%, 50%, 75%, and 100% were placed on the surface of NA media, which already contained S.epidermidis bacteria smear, positive control (+) used was clindamycin paper disc and 2% DMSO was used as negative control (-). A Paper disc containing the extract was inserted into the surface of the agar media. Then incubated at 37°C for 1x24 hours. After that, a clear zone's diameter as a zone of inhibition was measured with vernier callipers. Treatment was replicated four times (Kursia et al., 2016).

3. Results and Discussion

3.1.1. Sappan Wood Extract Yield

Results of extract obtained from a ratio of 1:10 with a total of sappan wood simplicial powder 400 g in 4000 mL of methanol solvent, a thick brownish red extract of 22 g with a yield of 5.5%.

3.1.2. Phytochemical Screening

The phytochemical test of sappan wood showed positive results for alkaloids, flavonoids, tannins and polyphenols, and triterpenoids but negative results for saponins. Based on the results of phytochemical screening, it was found that sappan wood methanol extract contained several bioactive compounds, as can be seen in Table 1.

Fig 1. Sappan wood, A: Simplicia; B: Maceration Results; C: Thick Extract

Antibacterial Activity Test of Sappan Wood Methanol Extract

An antibacterial activity test was conducted to determine the antibacterial inhibition ability of sappan wood extract against *S. epidermidis* bacteria, using various concentrations of extracts. The positive control was clindamycin 2µg antibiotic, while the negative control was DMSO 2%. The inhibition zone values obtained are shown in Figure 3.

Table 1. Phytochemical screening results

Phytochemical Test	Description	Result
Alkaloids 1. Mayer 2. Dregendrof	 Yellow precipitate was formed Orange precipitate was formed	 Positive Positive
Flavonoids	Clear orange foam was formed	Positive
Phenolics : 1. FeCl3 2. Gelatin	 Bluish-black colour was formed White precipitate was formed	 Positive Positive
Terpenoid	Brown ring is formed	Positive
Saponins	Unstable foam was formed	Negative

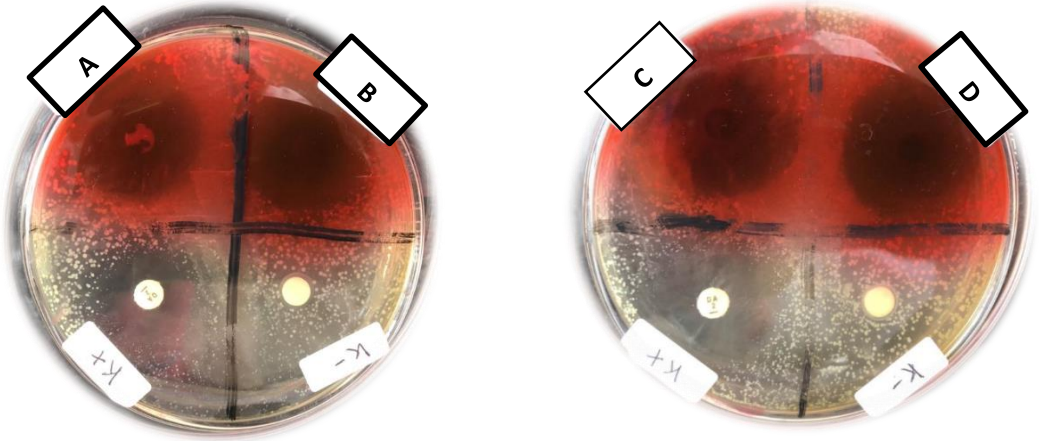

Fig 2. Antibacterial activity of Sappan wood methanol extract with concentration: A. 25%, B: 50%, C: 75%, D: 100%.

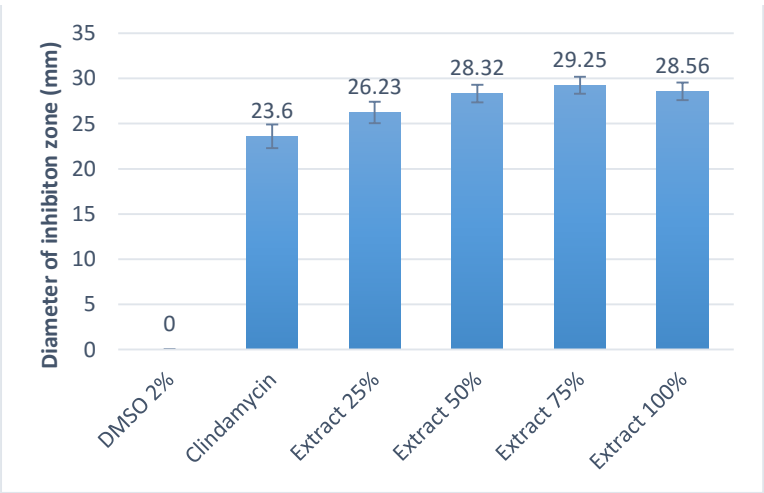

Fig 3. Antibacterial activity of sappan wood methanol extract

In this study, the antibacterial activity of sappan wood methanol extract against *S. epidermidis* could be seen by observing the presence or absence of bacterial growth around the paper discs (Figure 2). The concentration of sappan wood methanol extract was made with several concentrations (25%, 50%, 75% and 100%). This difference in concentration aimed to see how much difference in concentration can inhibit S. epidermidis bacteria.

Based on the study's results, it could be seen that sappan wood methanol extract has antibacterial inhibition against *S. epidermidis*. It could be seen from the presence of an inhibition zone formed in the diffusion test. Average inhibition zones produced in the antibacterial activity test were all concentrations of sappan wood methanol extract with concentrations of 25%, 50%, 75%, and 100% had very strong inhibition abilities against *S. epidermidis* bacteria which, according to (Davis & Stout, 1971), if the result of inhibition zone more than 20 mm it was included in the very strong category. All sappan wood methanol extract concentrations in this test had an inhibition zone > 20 mm. The highest average inhibition zone was produced at a concentration of 75%, 29.25 ± 0.95743 mm, followed by a 100% concentration of 28.56 ± 0.97072 mm, and a 50% concentration had an inhibition zone of 28.32 ± 0.97340. mm, and a concentration of 25% had an inhibition zone of 26.23 ± 1.18348 mm.

The diameter of the inhibition zone in general antibacterial activity test using extract tends to increase along the extract concentration. In this test, the average diameter of the inhibition zone obtained at concentrations of 75% and 100% gave an inhibitory zone of 29.25 ± 0.95743 mm and 28.56 ± 0.97072 mm, which means that the inhibition zone decreased. However, in this study, it was not as usual because there was a decrease in the inhibition zone at higher concentrations. These results prove that the increased concentration of sappan wood extract was not directly proportional to the increase in the inhibition zone for developing *S. epidermidis* bacteria. According to Davis & Stout, (1971) diameter of the inhibition zone formed did not always increase in proportion to the increase of extract concentration. It was due to differences in the diffusion rate of antibacterial compounds on agar media, and different types and concentrations of antibacterial compounds would give different diameters of inhibition zones at certain times.

Based on research (Batubara et al., 2009) stated that 50% methanol and ethanol extract of *C. sappan* showed low MIC/MBC values against *P. acne*. The research (2014) stated that the MIC/MBC value was 15.6/31.3 g/mL by isolating and identifying brazilin compounds. Research by Srinivasan et al., (2012) stated that the ethanol extract of *C. sappan* had antibacterial activity in *S. aureus* (31.0 ± 2.7 mm). Research by Xu & Lee, (2004), brazilin compounds in *C. sappan* were able to act as an antibacterial against *S. epidermidis* (MIC 8 g/ml) and *S. aureus* (MIC 16 g/mL). Research conducted by Cahyaningtyas et al., (2019), where in this study, the sample used was ethanol extract of sappan wood against *S. aureus* bacteria with concentrations of 25%, 50%, 75%, and 100% was able to inhibit *S. aureus* bacteria growth in the category very strong.

The antibacterial power of sappan wood methanol extract was inseparable from the presence of active substances which were thought to act as antibacterial in sappan wood methanol extract. The inhibition zone formed in the antibacterial activity test of sappan wood methanol extract was thought to be due to the presence of compounds as antibacterial from sappan wood extract that was able to inhibit bacterial growth. Currently, the research is also making efforts to identify phytochemical constituents analysis, and the results showed the presence of alkaloids, tannins, phenols, saponins and flavonoids and these phytochemical constituents were previously reported with some biological properties of phenolic compounds.

Flavonoids represent another bioactive compound class that inhibits bacterial growth by inhibiting DNA gyrase, disrupting cytoplasmic membrane function and inhibiting energy metabolism (Cushnie et al., 2016; Cushnie & Lamb, 2005; Nirmal et al., 2015). Phenol compounds also play an important role in inhibiting the growth of bacterial cells by the disrupted cytoplasmic membrane of bacterial cells, then causes changes in membrane permeability and finally causes leakage of constituents cytoplasmic (Johnston et al., 2003; Nirmal et al., 2015; Nirmal & Panichayupakaranant, 2014; Pattananandecha et al., 2022). Sappan wood plants could be an alternative therapy in acne treatment.

4. Conclusion

Sappan wood methanol extract (*Caesalpinia sappa*n L.) with UAE extraction method can inhibit the growth of *Staphylococcus epidermidis* bacteria very strongly at a concentration of 25%, 50%, 75%

and 100%. The phytochemical test of sappan wood showed positive results for alkaloids, flavonoids, tannins and polyphenols, and triterpenoids but negative results for saponins. Antibacterial activity of sappan wood methanol extract against Staphylococcus epidermidis gave the highest inhibitory power in the extract with a concentration of 75% with an average inhibition zone diameter of 29.25 ± 0.95743 mm and the lowest inhibitory power in the extract with a concentration of 25% with an average diameter of 26.23 ± 1.18348 inhibition.

Author Contributions: Waro Atunnisa and Irfan Zamzani wrote the first manuscript; Irfan Zamzani and Siti Nashihah analyzed the data; all authors interpreted the data and agreed to the final version of the manuscript. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests

The authors declare no conflict of interest.

Acknowledgment

Not available.

References

Al Kadri, M. F., Sunarni, T., Pamudji, G., & Zamzani, I. (2019). Aktivitas antioksidan ekstrak etanol daun Pelawan (Tristaniopsis obovate. Benn) dengan metode penangkapan radikal bebas 2,2'-difenil-1-pikrilhidrazil. *Journal of Current Pharmaceutical Science*, *2*(2), 167–172.

Arista, Y., Kumesan, N., Yamlean, P. V. Y., & Supriati, H. S. (2013). Formulasi dan uji aktivitas gel antijerawat ekstrak Umbi Bakung (Crinum Asiaticum L.) terhadap Bakteri Staphylococcus Aureus secara in vitro. *Pharmacon Jurnal Ilmiah Farmasi – UNSRAT*, *2*(02), 2302–2493.

Batubara, I., Mitsunaga, T., & Ohashi, H. (2009). Screening antiacne potency of Indonesian medicinal plants: Antibacterial, lipase inhibition, and antioxidant activities. *Journal of Wood Science*, *55*(3), 230–235. https://doi.org/10.1007/s10086-008-1021-1.

Cahyaningtyas, D. M., Puspawati, N., & Binugraheni, R. (2019). Uji aktivitas antibakteri ekstrak etanolik kayu Secang (Caesalpinia sappan L.) terhadap Staphylococcus aureus. *Biomedika*, *12*(2), 205–216. https://doi.org/10.31001/biomedika.v12i2.614.

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002.

Cushnie, T. P. T., O'Driscoll, N. H., & Lamb, A. J. (2016). Morphological and ultrastructural changes in bacterial cells as an indicator of antibacterial mechanism of action. *Cellular and Molecular Life Sciences*, *73*(23), 4471–4492. https://doi.org/10.1007/s00018-016-2302-2.

Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. *Applied Microbiology*, *22*(4), 659–665. https://doi.org/10.1128/aem.22.4.666-670.1971.

Dharmayanti, N. M. D., & Arjita, I. P. D. (2019). Uji daya hambat ekstrak batang kayu Secang (Caesalpinia Sappan L.) terhadap bakteri Escherichia Coli. *Jurnal Kedokteran*, *4*(1), 685. https://doi.org/10.36679/kedokteran.v4i1.60.

Johnston, M. D., Hanlon, G. W., Denyer, S. P., & Lambert, R. J. W. (2003). Membrane damage to bacteria caused by single and combined biocides. *Journal of Applied Microbiology*, *94*(6), 1015–1023. https://doi.org/10.1046/j.1365-2672.2003.01923.x.

Kursia, S., Lebang, J. S., Taebe, B., Burhan, A., Rahim, W. O. ., & Nursamsiar. (2016). Uji aktivitas antibakteri ekstrak etilasetat daun sirih hijau (Piper betle L.) terhadap Bakteri Staphylococcus epidermidis. *Indonesian Journal of Pharmaceutical Science and Technology*, *3*(2), 72–77.

Neswati, N., & Ismanto, S. D. (2018). Ekstraksi komponen bioaktif serbuk kayu Secang (Caesalpinia sappan, L) dengan metode ultrasonikasi. *Jurnal Teknologi Pertanian Andalas*, *22*(2), 187. https://doi.org/10.25077/jtpa.22.2.187-194.2018.

Nirmal, N. P., Rajput, M. S., Prasad, R. G. S. V., & Ahmad, M. (2015). Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. *Asian Pacific Journal of Tropical Medicine*, *8*(6), 421–430. https://doi.org/10.1016/j.apjtm.2015.05.014.

Nirmal, N., & Panichayupakaranant, P. (2014). Anti-Propionibacterium acnes assay-guided purification of brazilin and preparation of brazilin rich extract from Caesalpinia sappan heartwood. *Pharmaceutical Biology*, *52*(9), 1204–1207. https://doi.org/10.3109/13880209.2014.884607.

Pattananandecha, T., Apichai, S., Julsrigival, J., Ogata, F., Kawasaki, N., & Saenjum, C. (2022). Antibacterial activity against foodborne pathogens and inhibitory effect on anti-inflammatory mediators ' production of Brazilin-Enriched extract from Caesalpinia sappan Linn. *Plants*, *11*, 1–10. https://doi.org/https://doi.org/ 10.3390/plants11131698.

Retnaningsih, A., Primadiamanti, A., & Febrianti, A. (2019). Uji daya hambat ekstrak etanol daun Ungu (Graptophyllum pictum (L.) GRIFF) terhadap bakteri Staphylococcus epidermidis dan Propionibacterium acnes penyebab jerawat dengan metode cakram. *Jurnal Analisis Farmasi*, *4*(1), 1–9.

Rosidah, M. S., Lambui, O., & Suwastika, I. N. (2018). Ekstrak daun tumbuhan Macaranga tanarius (L .) M . A menghambat laju pertumbuhan bakteri Staphylococcus epidermidis. *Journal of Science and Technology 1*, *7*(1), 64–70.

Srinivasan, R., selvam, G. G., Karthik, S., Mathivanan, K., Baskaran, R., Karthikeyan, M., Gopi, M., & Govindasamy, C. (2012). In vitro antimicrobial activity of Caesalpinia sappan L. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1 SUPPL.), S136–S139. https://doi.org/10.1016/S2221-1691(12)60144-0.

Suryana, S., Nuraeni, Y. Y. A., & Rostinawati, T. (2017). Aktivitas antibakteri ekstrak etanol dari lima tanaman terhadap Bakteri Staphylococcus epidermidis dengan metode Mikrodilusi M7 – A6CLSI. *Indonesian Journal of Pharmaceutical Science and Technology*, *4*(1), 1. https://doi.org/10.15416/ijpst.v4i1.8982.

Wijayati, N., Astutiningsih, C., Mulyati, S., & Artikel, I. (2014). Transformasi α-Pinena dengan Bakteri Pseudomonas aeruginosa ATCC 25923. *Biosaintifika: Journal of Biology & Biology Education*, *6*(1), 24–28. https://doi.org/10.15294/biosaintifika.v6i1.2931.

Xu, H. X., & Lee, S. F. (2004). The antibacterial principle of Caesalpina sappan. *Phytotherapy Research*, *18*(8), 647–651. https://doi.org/10.1002/ptr.1524