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Antibacterial activities of water extract of *Vernonia amygdalina* Delile leaves

[1,*]Mahatir, M., [2]Effendy, D.L., [2]Henni, C. and [2]Denny, S.

[1]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara. Jl. Tri Dharma No.5, Medan, North Sumatera, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara. Jl. Tri Dharma No.5, Medan, North Sumatera, Indonesia*

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Abstract

Staphylococci are common bacterial colonizers of the skin and mucous membranes of humans and other mammals. *Staphylococcus epidermidis* in particular is the most frequently isolated species from human epithelia. It colonizes predominantly the axillae, head, and nares. One type of medicinal plant that is nutritious for health is African leaves (*Vernonia amygdalina* Delile). *Vernonia amygdalina* extract showed the presence of some compounds which are rich in those constituents. The phytochemical screening of the extracts showed variation in their phytochemical constituents with the presence and or absence of some components. The presence of glycosides, alkaloids, and flavonoids was believed to exhibit the antibiotic properties of *V. amygdalina* leaves and confirmed their antimicrobial efficacy against selected pathogens. The concentration of 50 mg/mL up to a concentration of 1.56 mg/mL can inhibit the growth of *S. epidermidis*, the Minimum Inhibitory Concentration (MIC) itself is at the smallest concentration that has been able to inhibit bacterial growth and the number of bacteria is < 10 colonies. *Staphylococcus epidermidis* at a concentration of 25 mg/mL, absorbance values at wavelengths of 260 nm and 280 nm were higher than the absorbance values of *S. epidermidis* at a concentration of 12.5 mg/mL. The absorbance value at wavelengths of 260 nm and 280 nm will increase with the higher concentration of the extract, this indicates the presence of cell leakage observed in the presence of protein and nucleic acid leakage. *S. epidermidis* biofilm inhibition test shows that the results of the absorbance or OD (Optical Density) measurement in the biofilm inhibition test using the ethanol extract of *V. amygdalina* Delile, the OD value at a concentration of 25 mg/mL ie 0.149±0.004 showed a biofilm inhibition result of 62.37% greater than the concentration of 12.5 mg/mL which is 0.318±0.003 with a biofilm inhibition result of 19.52%, this indicates that the higher the concentration of the extract, the smaller the biofilm formation of *S. epidermidis* bacteria.

1. Introduction

Analysis of the *Staphylococcus epidermidis* genome indicated that the species is well equipped with genes assumed to protect from the harsh conditions encountered in its natural habitat (Roger *et al*., 2009; Chu *et al*., 2009). Specific antibiotic-resistance genes are widespread in *S. epidermidis*. Most notably, resistance to methicillin as an antibiotic of the first choice against staphylococcal infections is at 75–90% among hospital isolates of *S. epidermidis*, which is even higher than the corresponding rate for *Staphylooccus aureus* (40–60%) (Diekema *et al*., 2001)

The use of antibacterial drugs for the treatment of infectious diseases caused by bacteria is now quite a lot, but the problem faced now is the occurrence of side effects for its users, such as diarrhoea, allergies, other toxic hazards, as well as the high consumption of maintenance costs. The number of cases of infection due to bacteria, the emergence of side effects of using antibacterial drugs, as well as the high consumption of treatment costs indicate the need for research to develop new antibacterial, especially from natural ingredients (Osman, 2009).

Herbal plants are still the main choice used in medicine in some parts of the world (Al-Rubiay *et al*., 2008). Secondary metabolites produced by plants have

*Corresponding author.
Email: *mahatir.muhammad@usu.ac.id*

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been recognized to have many pharmacological activities. Diseases that are often treated with herbal plants are infections caused by bacteria (Borade *et al*., 2011).

One type of medicinal plant that is nutritious for health is African leaves (*Vernonia amygdalina* Del.). African leaves have properties in curing various diseases and are very easy to grow so they are easy to find. African leaves contain flavonoids, tannins, saponins, and terpenoids which can kill parasites that cause malaria, antiamoeba, anti-tumour, and antimicrobial. In addition, African leaves have benefits for diabetes, diarrhoea, malaria, stabilize blood pressure, help cure insomnia, help prevent stroke, prevent cancer, and prevent heart disease (Ijeh and Ijeke, 2011).

2. Materials and methods

2.1 Materials

Fresh leaves of *Vernonia amygdalina* Delile were collected from the Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.

2.2 Preparation of extraction

Vernonia amygdalina Delile leaves were dried at 45° C temperature and powdered. Simplicia powder of *V. amygdalina* Delile leaves (500 g) was extracted using ethanol absolute with the reflux method (Dalimunthe *et al*., 2018; Harahap *et al*., 2018; Hasibuan *et al*., 2020).

2.3 Determination of minimum inhibitory concentration

Antibacterial activities of extracts were examined by agar well plate diffusion assay method, minimum inhibitory concentration (MIC). The examination was carried out based on the previous procedure that used Mueller Hinton Agar (MHA) as test media and pure bacteria culture (*S. epidermidis*) with several treatment modifications. Negative blanks used a mixture of dimethylsulfoxide (DMSO, Merck®) (Boateng and Diunase., 2015).

2.4 Determination leakage of cellular metabolites

Determination leakage of cellular metabolites based on the previous literature with several modifications using a spectrophotometer (ultraviolet-visible Spectrophotometer (UVS) using Thermo Fisher Scientific. A total of 5 mL of MHB included bacterial inoculum suspension by 1 mL, then added extract and negative control of 1 mL each. Then incubated in an incubator of 35±20°C for 24 hrs under aerobic conditions. After that, the solution was centrifuged at 3500 rpm for 30 mins, then the supernatant was separated from the cell precipitate. The absorbance of the supernatant was immediately measured with a spectrophotometer. The procedure was carried out six repetitions (Miksusanti *et al*., 2008).

2.5 Determination of biofilm

Extracts with different concentrations were put into sterile polystyrene microtiter plates; previously, then wells were microtiter plates containing 2 mL MHB medium, inoculated, samples were incubated at 37°C for 18 hrs. After incubation, 1000 µL saline (0.85% NaCl) was added to each well. Then, the wells were washed three times with sterile distilled water, and the plates were dried for 45 mins before quantitatively testing the biofilm. Growth and quantification of the biofilms were evaluated using the crystal violet stain method and measured the absorption was at 575 nm (Nosrati *et al*., 2018).

3. Results and discussion

3.1 Minimum inhibitory concentration

In Table 1 it can be seen that the concentration of 50 mg/mL up to a concentration of 1.56 mg/mL can inhibit the growth of *S. epidermidis*, the Minimum Inhibitory Concentration (MIC) itself is the smallest concentration that has been able to inhibit bacterial growth and the number of bacteria is < 10 colonies. MIC is the minimum concentration of antimicrobial substances that can inhibit bacterial growth after 24 hrs of incubation and no known bacterial colonies grow by observing the number of bacterial colonies that grow (Tortora *et al*., 2010).

3.2 Leakage of cellular metabolites

In Table 2, *S. epidermidis* at a concentration of 25 mg/mL, absorbance values at wavelengths of 260 nm and 280 nm were higher than the absorbance values of *S. epidermidis* at a concentration of 12.5 mg/mL. The absorbance value at wavelengths of 260 nm and 280 nm will increase with the higher concentration of the extract, this indicates the presence of cell leakage observed in the presence of protein and nucleic acid leakage. An increase in the absorbance value in the measured cells indicates an increase in the number of cell contents removed from the cell. The components of cell contents that leak out of the cell that can be measured at a wavelength of 260 nm

Table 1. The minimum inhibitory concentration of *Staphylococcus epidermidis.*

Extract	Inhibition zone diameter (Concentration mg/mL)					
	50	25	12.5	6.25	3.125	1.56
Vernonia amygdalina Delile	24.27±0.09	21.40±0.15	18.23±0.15	14.37±0.09	12.37±0.12	11.10±0.10

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Table 2. Leakage of cellular metabolites of *Staphylococcus epidermidis.*

Staphylococcus epidermidis	Leakage of cellular metabolites		
	260 nm	280 nm	Ratio 260/280
25 mg/mL	0.268±0.006	0.331±0.004	0.810±0.009
12.5 mg/mL	0.176±0.005	0.252±0.006	0.701±0.008

are DNA including purines, pyrimidines, and ribonucleotides, while at a wavelength of 280 nm can measure tyrosine and tryptophan. Methods to correct for the presence of nucleic acids can be developed by measuring the absorbance ratios at 280 and 260 nm. This ratio can be used to estimate the amount of protein that also contains nucleic acids. Microbes are sensitive if bacterial growth and microbial death are inhibited. The mechanism of inhibition or death of microbes on exposure to active compounds can be identified by the presence of cell leakage, metabolic disorders, and changes in cell morphology (Park and Park, 2003).

3.3 Biofilm

In Table 3, *S. epidermidis* biofilm inhibition test shows that the results of the absorbance or OD (Optical Density) measurement in the biofilm inhibition test using the ethanol extract of *V. amygdalina* Delile, the OD value at a concentration of 25 mg/mL, i.e., 0.149±0.004 showed a biofilm inhibition result of 62.37% greater than the concentration of 12.5 mg/mL which is 0.318±0.003 with a biofilm inhibition result of 19.52%, this indicates that the higher the concentration of the extract, the smaller the biofilm formation of *S. epidermidis* bacteria. The mechanism of inhibition of biofilm growth can be by penetrating the bacterial cell wall so that it can interfere with communication signals (Quorum sensing) between bacteria that play a role in biofilm formation or inactivate genes in bacteria that trigger EPS synthesis (Aenderek *et al*., 2005).

Table 3. Biofilm inhibition.

Staphylococcus epidermidis	Biofilm		
	Blank	Optical density	Percentage (%)
25 mg/mL	0.396	0.149±0.004	62.37±1.14
12.5 mg/mL		0.318±0.003	19.52±0.72

Verona amygdalina extract showed the presence of some compounds which are rich in those constituents. The phytochemical screening of the extracts showed variation in their phytochemical constituents with the presence and or absence of some components. The presence of glycosides, alkaloids, and flavonoids was believed to exhibit the antibiotic properties of *V. amygdalina* leaves and confirmed their antimicrobial efficacy against selected pathogens. This study suggested that plant's phytochemical constituents that can either inhibit the growth of pathogens or kill them be considered potential candidates for developing new antimicrobial drugs (Ashok and Ramaswamy, 2014)

Based on the results of research that have been carried out through phytochemical screening tests, data obtained in the form of compounds contained in the ethanol extract of *V. amygdalina* namely flavonoids, saponins, tannins, steroids, and triterpenoids, where these compounds have different anti-bacterial mechanisms (Atangwho *et al*., 2013).

Flavonoids work as antibacterial because of their ability to form complex compounds with extracellular and soluble proteins (Mercy *et al.,* 2013). In addition, another mechanism of the flavonoid group in inhibiting bacterial growth is the inhibition of the biofilm layer on bacteria.

The mechanism of saponins as an antibacterial is by lowering the surface tension, resulting in increased permeability of cell leakage and resulting in the release of intracellular compounds (Nuria *et al*., 2009). This compound diffuses through the outer membrane and the fragile cell wall, then binds to the cytoplasmic membrane and disrupts and reduces its stability (Cavalieri *et al*., 2005).

The mechanism of tannin as an antibacterial is related to the inhibition of bacterial enzymes, where the transcriptase and DNA topoisomerase enzymes cannot be formed. In addition, tannins have an antibacterial activity that is associated with inactivating microbial cell adhesins as well as inactivating enzymes and disrupting protein transport (Sari and Sari, 2011). To maintain their survival, microbial cells need to synthesize proteins that take place on the ribosomes, protein disturbances will be very fatal, and anti-microbial with a mechanism of action like this has strong antibacterial power.

The mechanism of steroids as antibacterials is related to lipid membranes and sensitivity to steroid components that cause leakage in liposomes. Steroids can interact with cell phospholipid membranes which are permeable to lipophilic compounds, causing decreased membrane integrity and cell membrane morphology to change which causes cell brittleness and lysis (Madduluri *et al.*, 2013).

The mechanism of triterpenoids as an antibacterial is to react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond, resulting in the destruction of the porin.

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Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall which will result in the bacterial cell being deprived of nutrients so that bacterial growth is inhibited or dies. The mechanism of action of the triterpenoid group of compounds is also related to the inhibition of glycolysis, fatty acid synthesis, amino acid synthesis, and the synthesis of peptidoglycan (Park *et al*, 2007).

4. Conclusion

Vernonia amygdalina Delile water extract has antibacterial activity ability in minimum inhibitory concentration, leakage of cellular metabolites, and biofilm inhibition against *S. epidermidis* bacteria.

Conflict of interest

The authors declare no conflict of interest.

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References

Aenderek, S., Diggle, S., Song, Z., Hoiby, N., Cornelis, P., Williams, P. and Camara, M. (2005). The MexGHI-OpmD multidrug efflux pump controls growth, antibiotic susceptibility and virulence in Pseudomonas aeruginosa via 4-quinolonedependent cell to- cell communication. *Microbiology*, 151(4), 1113-1125. https://doi.org/10.1099/mic.0.27631-0

Al-Rubiay, K.K., Jaber, N.N., Al-Mhaawe, B.H. and Alrubaiy, L.K. (2008). Antimicrobial Efficacy of Henna Extracts. *Oman Medical Journal*, 23(4), 253-256.

Ashok K.R. and Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences,* 3(1), 395-406.

Atangwho, I.J., Egbung, G.E., Ahmad, M., Mun, F. and Mohd, Z. (2013). Antioxidant versus anti-diabetic properties of leaves from *Vernonia amygdalina* Del. growing in Malaysia. *Food Chemistry*, 141(4), 3428-3434. https://doi.org/10.1016/j.foodchem.2013.06.047

Boateng, J. and Diunase, K.N. (2015). Comparing the Antibacterial and Functional Properties of Cameroonian and Manuka Honeys for Potential Wound Healing-Have We Come Full Cycle in Dealing with Antibiotic Resistance. *Molecules*, 20 (9), 16068. https://doi.org/10.3390/molecules200916068

Borade, A.S., Kale, B.N. and Shete, R.V. (2011). A Phytopharmacological Review on *Lawsonia inermis* (Linn.). *International Journal of Pharmacy and Life Sciences*, 2(1), 536-541.

Cavalieri, S.J., Rankin, I.D., Harbeck, R.J., Sautter, R.S., McCarter, Y.S., Sharp, S.E., Ortez, J.H. and Spiegel, C.H. (2005). Manual of Antimicrobial Susceptibility Testing. USA: American Society for Microbiology.

Chu, V.H., Miro, J.M., Hoen, B., Cabell, C.H., Pappas, P.A., Jones, P., Stryjewski, M.E., Anguera, I., Braun Muñoz, S.P., Commerford, P., Tornos, P., Francis, J., Oyonarte, M., Selton-Suty, C., Morris, A.J., Habib, G., Almirante, B., Sexton, D.J., Corey, G.R. and Fowler, V.G. (2019). Coagulase-negative staphylococcal prosthetic valve endocarditis--a contemporary update based on the International Collaboration on Endocarditis: prospective cohort study. *Heart*, 95(7), 570–576. https://doi.org/10.1136/hrt.2008.152975

Dalimunthe, A., Hasibuan, P.A.Z., Silalahi, J., Sinaga, S.F. and Satria, D. (2018). Antioxidant activity of alkaloid compounds from *Litsea cubeba* Lour. *Oriental Journal of Chemistry*, 34(2), 1149-1152. http://doi.org/10.13005/ojc/340270

Diekema, D.J., Pfaller, M.A., Schmitz, F.J., Smayevsky, J., Bell, J., Jones, R.N. and Beach, M. (2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clinical Infectious Diseases*, 32(Suppl. 2), S114–S132. https://doi.org/10.1086/320184

Harahap, U., Hasibuan, P.A.Z., Sitorus, P., Arfian, N. and Satria, D. (2018). Antimigration activity of an ethylacetate fraction of *Zanthoxylum acanthopodium* DC. fruits in 4T1 breast cancer cells. *Asian Pacific Journal of Cancer Prevention*, 19(2), 565-569. https:// doi.org/10.22034%2FAPJCP.2018.19.2.565

Hasibuan, P.A.Z., Harahap, U., Sitorus, P. and Satria, D. (2020). The anticancer activities of *Vernonia amygdalina* Delile. Leaves on 4T1 breast cancer cells through phosphoinositide-3-kinase (PI3K pathway). *Heliyon*, 6(7), e04449. https://doi.org/10.1016/j.heliyon.2020.e04449

Ijeh, I.L. and Ejike, C.E.C.C. (2011). Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. *Journal of Medicinal Plant Research*, 5(7), 1051-1061. https://doi.org/10.5897/

JMPR.9000004

Madduluri, S., Rao, K.B. and Sitaram, B. (2013). In vitro evaluation of antibacterial activity of five indigenous plants extract against five bacterial pathogens of human. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(4), 679-684.

Mercy, N., Jemmy, A. and Vanda, S. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (*Pometia pinnata)* terhadap Bakteri *Staphylococcus aureus* secara *In vitro*. *Journal MIPA UNSRAT Online*, 2, 128-132. https://doi.org/10.35799/jm.2.2.2013.3121 [In Bahasa Indonesia].

Miksusanti, B.S.L., Jenie, B.P., Priosoeryanto, R., Syarief, G.T. and Rekso. (2008). Mode of Action Temu Kunci (*Kaempferia pandurata*) Essential Oil on *E. coli* K1.1 Cell Determined by Leakage of Material Cell and Salt Tolerance Assays. *HAYATI Journal of Biosciences*, 15(2), 56-60. https:// doi.org/10.4308/hjb.15.2.56

Nosrati, M., Behbahani, M., Mohabatkar, H. and Shakeran, Z. (2018). Antibacterial and Antibiofilm Activities of *Prangos acaulis* Born Extract Against *Streptococcus mutans*: An *in Silic*o and *In Vitro* Study. *Journal of Herbmed Pharmacology*, 7(3), 176 – 184. https://doi.org/10.15171/jhp.2018.29

Osman, H. (2009). Antioxidant Activity and Phenolic Content of *Syzygium aquea* Molecules. Malaysia: Bandar Sunway Publishing. https://doi.org/10.3390/ molecules14030970

Park, P.K., Kim, E.Y. and Chu, K.H. (2007). Chemical disruption of yeast cells for the isolation of carotenoid pigments. *Separation and Purification Technology*, 53(2), 148-152. https://doi.org/10.1016/ j.seppur.2006.06.026

Park, P.S. and Park, H.W. (2003). Inactivation kinetics of food poisoning microorganisms by carbon dioxide and high hydrostatic pressure. *Journal of Food Science*, 68(3), 976-81. https://doi.org/10.1111/ j.1365-2621.2003.tb08273.x

Roger, K.L., Fey, P.D. and Rupp, M.E. (2019). Coagulase-negative staphylococcal infections. *Infectious Disease Clinics of North America*, 23(1), 73–98. https://doi.org/10.1016/j.idc.2008.10.001

Sari, F.P. and Sari, S.M. (2011). Ekstraksi Zat Aktif Antimikroba dari Tanaman Yodium (*Jatropha multifida* Linn) sebgai Bahan Baku Alternatif Antibiotik Alami. Semarang, Indonesia: Fakultas Teknik Universitas Diponegoro. [In Bahasa Indonesia].

Tortora, G.J., Funke, B.R. and Case, C.L. (2010). Microbiology an Introduction. San Francisco, USA: Addison Wesley Longman Inc.

https://doi.org/10.26656/fr.2017.7(3).832