JURNAL PENDIDIKAN KIMIA (JPKIM)
ISSN: 2085-3653| e-ISSN: 2549-3116

J. Pendidik. Kim 2023, 15(1), 18 - 22
DOI 10.24114/jpkim.v15i1.42154

Research Article

In vitro test of antibacterial activity causing inhibition of wound healing from ethanol extract of nangka (*Artocarpus heterophyllus*, Lam.) leaf

Esther Siringo-ringo[1], Indah Yani Tambunan[1], Mastiur Julianti Butar-butar[1], Kasta Gurning[2], Iksen[3]

[1]*Departement of Midwifery, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan 20141, Indonesia*
[2]*Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan 20141, Indonesia*
[3]*Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

Received 04 January 2023 ♦ **Revised** 01 April 2023 ♦ **Accepted** 08 April 2023

Citation: Siringo-ringo, E., Tambunan, I. Y., Butar-butar, M. J., Gurning, K., & Iksen, I. (2023). In vitro test of antibacterial activity causing inhibition of wound healing from ethanol extract of nangka (Artocarpus heterophyllus, Lam.) leaf. Jurnal Pendidikan Kimia (JPKIM), 15(1), 18–22. https://doi.org/10.24114/jpkim.v15i1.42154

Keywords

Antibacterials
Escherichia coli
Nangka leaf
Staphylococcus aureus
Wound healing

Corresponding author:
E-mail: kastagurning@gmail.com
(Kasta Gurning)

Abstract

Nangka (*Artocarpus heterophyllus*, Lam.) is a plant that is rich in nutrients and contains phytochemicals that can be used in various fields, especially in the health sector. Traditionally, every part of this plant has been widely used for its anticarcinogenic, antimicrobial, antifungal, anti-inflammatory, wound healing, and hypoglycemic effects. Antibacterials are an important part of drug preparations to accelerate wound healing. Antibiotics inhibit the occurrence of infection and stimulate the process of forming fibroblast cells for wound closure. Bacteria that are often found in wounds and inhibit the acceleration of wound healing are *Staphylococcus aureus* and *Escherichia coli*. The purpose of this study was to test the activity of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) in vitro on *E. coli* and *S. aureus* bacteria. The extraction method used was maceration with ethanol solvent and in vitro antibacterial activity testing using the paper disc method, as well as variations in extract concentrations, namely 12.5%, 25%, 50%, 75%, chloramphenicol as a positive control, and 5% DMSO as a negative control. The results showed that the ethanol extract contained various secondary metabolites, namely alkaloids, tannins, saponins, steroids/triterpenoids, and flavonoids. The ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) has strong category potential as an antibacterial against *E. coli* and *S. aureus*.

OpenAcces

Introduction

Bacteria are one of the factors that influence how quickly or slowly a wound heals. The presence of bacteria in the wound will cause infection, which will lead to serious complications that can interfere with health (Zhou et al. 2018). Wounds that are open and moist are factors that support bacterial colonization and infection in the wound, which results in inflammation and leads to delays in wound healing. Therefore, preventing colonization or bacterial infection of the wound is important for accelerating wound healing (Chen et al. 2020).

Types of bacteria that cause delays in healing and cause wound infections are *Staphylococcus aureus* (Schneider et al. 2007) and *Escherichia coli* (Laguliga et al. 2021). *E. coli* bacteria, including gram-negative bacteria, and *S. aureus bacteria*, including gram-positive bacteria, can inhibit the process of fibroblast cell proliferation, which inhibits wound healing (Thapa et al. 2020).

The nangka plant, besides its abundant nutritional content, also has a class of bioactive compounds such as sapogenins, phenolics, carotenoids, flavonoids, volatile acid sterols, and tannins. The content of a class of bioactive compounds supports pharmacological activity, including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, antidiabetic, antioxidant and wound healing (Ranasinghe et al. 2019; Gupta et al. 2020). In general, a wound can be defined as a condition in which there is damage to body tissue, while an incised wound is generally defined as damage to the skin tissue due to a sharp object that makes the skin

surface injured and bleeds. Damage to the skin tissue structure can be limited to the epidermal layer, reach the dermis, or even injure the muscle layer (Sayogo et al. 2017). Based on the description above, this study aims to test the activity of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) in vitro, which was tested on *E. coli* and *S. aureus* bacteria.

Method

Preparation Sample

The samples of nangka leaf (*Artocarpus heterophyllus*, Lam.) used were taken from trees that had previously produced fruit in good and fresh condition. The process of sample preparation and extract preparation refers to standard preparation, which starts with washing the sample with running water, draining it, and then drying it in a dryer cupboard at 55°C. The dry sample is blended to increase the contact surface area with the solvent so that the extraction process becomes optimal. The extraction was carried out referring to the list of references used (Gurning et al. 2021; Situmorang et al. 2022).

Phytochemical Screening

A phytochemical screening was carried out to identify the secondary metabolite groups present in the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.). Phytochemical screening included alkaloids with Dragendroff's reagent, tannins with 1% $FeCl_3$ reagent, terpenoids and steroids with Liebermann Bouchard reagent, flavonoids with magnesium powder reagent and concentrated HCl (Shinoda-test), phenolics and polyphenols with 10% $FeCl_3$ reagent dissolved in ethanol, and saponins with Aquadest reagent (Gurning et al. 2022).

Test of Antibacteria Activity

A determination of antibacterial activity was carried out on *E. coli* and *S. aureus* bacteria. The method used in determining activity is the paper disc method. Preparation starts with sterilizing the tools and materials to be used, as well as rejuvenating bacteria, making media, making bacterial suspensions, and making variations in the concentration of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.), which will be tested. The media used were MHA agar media, 5% DMSO solvent, and chloramphenicol as a positive control. The concentration variations of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) used were 12.5%, 25%, 50%, and 75% which were measured in duplo. At room temperature (37°C), observations were made for 1x24 hours. Observations were made by measuring the diameter of the inhibition zone formed around the paper disc using a digital caliper (electronical digital caliper brand). The activity value index (AVI) was calculated using the following equation (Kuspradini et al. 2019; Nasri et al. 2022; Juwitaningsih et al. 2022):

$$\text{Activity Value Index (AVI)} = \frac{\textit{Inhibition zone of ethanol extract nangka Leaf}}{\textit{Inhibition zona of positive control}}$$

Results and Discussion

Preliminary Phytochemical Screening

The results of the phytochemical screening of the ethanol extract of nangka (*Artocarpus heterophyllus*, Lam.) leaf are presented in Table 1.

Table 1. Phytochemical Screening of Nangka Leaf Ethanol Extract Results

Secondary Metabolites	Reagent	Results
Alkaloids	Dragendrofft	+
Tannins	$FeCl_3$ 1%	+
Steroids/triterpenoids	Liebermann Bouchard	+ / +
Flavonoids	Mg $_{(s)}$ +HCl $_{(concentrat)}$	+
Phenolic/Polyphenol	$FeCl_3$ 10% at ethanol	+
Saponins	Foaming test	+

Description: + = positive or present

The results of the phytochemical screening performed on the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) showed a variety of secondary metabolites. The content of various secondary metabolites

that are possessed supports the utilization of various potential activities, in this case as antibacterial activity against bacteria that inhibit wound healing, namely *E. coli* and *S. aureus*. The content of secondary metabolites in various tannin groups has been reported to have antibacterial, antiviral, and antifungal activities (Tambunan et al. 2023; Silaban et al. 2022).

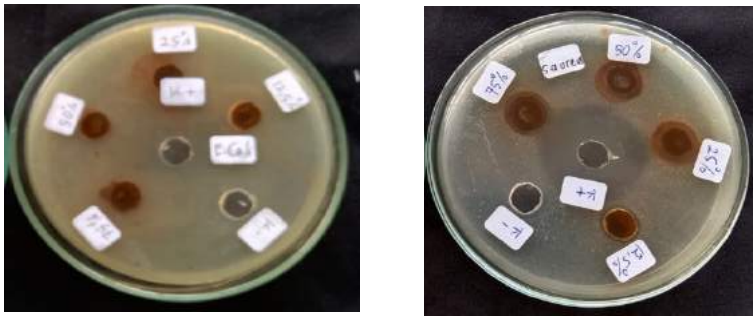
Antibacterial Activity Test

Antibacterial activity testing was carried out in vitro using the paper disc method, 5% DMSO as a negative control and chloramphenicol as a positive control. Antibacterial activity of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) against *E. coli* and *S. aureus*, which has been widely reported to have a role as an inhibitor of wound healing on the skin by inhibiting the proliferative process of fibroblast cells in wound closure (Thapa et al. 2020). The results of testing activity against bacteria can be seen in Table 2 and Fig.-1.

Table 2. Activity Test Results of Nangka Leaf Ethanol Extract Against Bacteria

Treatmen	*E. coli*		*S. aureus*	
	Inhibition Zone (mm)	AVI	Inhibition Zone (mm)	AVI
75%	16.20±0.42	0.53	16.70±0.28	0.53
50%	15.90±0.28	0.52	15.40±0.99	0.49
25%	15.10±0.14	0.49	14.10±1.56	0.45
12.5%	14.30±0.28	0.47	12.30±1.70	0.39
DMSO 5%	0.00	0.00	0.00	0.00
Chloramphenicol	30.60±0.71	1.00	31.60±2.12	1.00

The results are given as Mean±SD, n = 2; 5%DMSO as a negative control, and chloramphenicol as a positive control.



a b

Fig.-1. Inhibition zone of ethanol extract of nangka leaf against *E. coli* (a) and *S. aureus* (b) bacteria

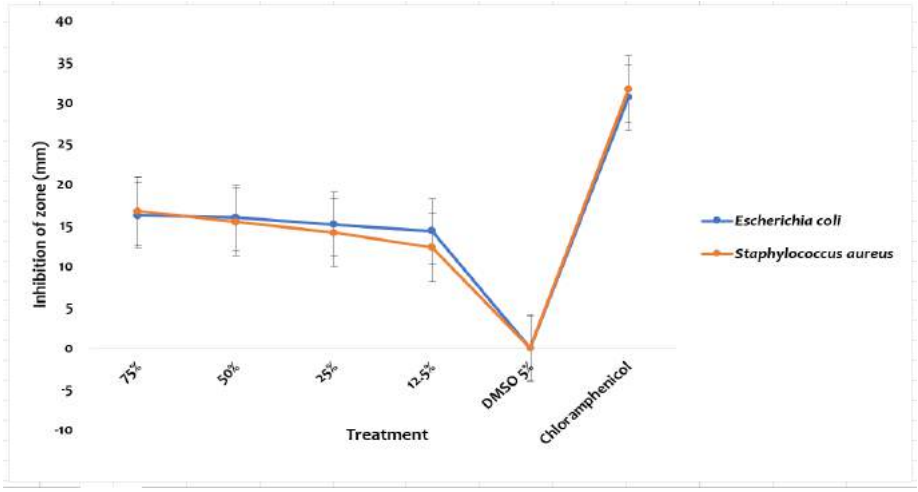


Fig.-2. Antibacterial Activity of Ethanol Extract of Nangka Leaf

The test results for the activity of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) against *E. coli* and *S. aureus* bacteria were included in the "strong" category. This is in accordance with the grouping of antibacterial activity categories, where the clear inhibition zone < 5 mm is included in the weak category, the clear inhibition zone of 5–10 mm is included in the moderate category, the clear inhibition zone of 10–20 mm is included in the strong category, and the clear inhibition zone > 20 mm is included in the very strong category (Sumilat, 2019). Besides the tannin content, which is reported to have potential as an antibacterial, the alkaloids, flavonoids, and saponins also have a role as an antibacterial (Nasri et al. 2022). The potential antibacterial activity of increasing the concentration of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) is directly proportional to the inhibitory activity against *E. coli* and *S. aureus* bacteria (Fig.-2), but the strength of the inhibition is not so significant with increasing concentrations.

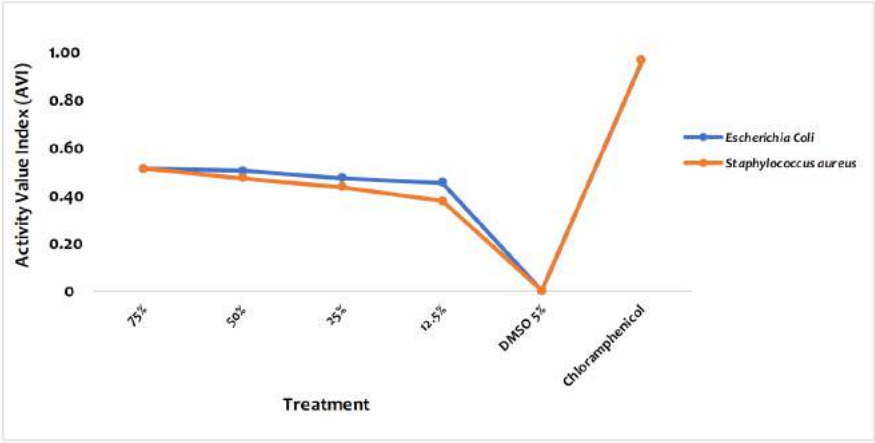


Fig.-3. Activity Value Index of Ethanol Extract of Nangka Leaf Against Positive Control

The potency of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam) against *E. coli* and *S. aureus* bacteria was not as strong as chloramphenicol as a positive control. Chloramphenicol is a class of antibiotic compounds that are commonly used as antibacterials because they are very strong and have a broad spectrum. Measurement of the activity index value to estimate the strength of the antibacterial activity of each extract variation against the positive control (Fig.-3). If the activity index value of the concentration variation of the ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam) is close to 1.00, it indicates that the concentration variation has the same strength as the positive control as an antibacterial (Kuspradini et al. 2019). The results of this study indicate the potential for activity to inhibit bacterial growth, so it is necessary to carry out further testing of the development of this research in the form of medicinal preparations for wounds such as ointments and tested in vivo.

Conclusion

The ethanol extract of nangka leaf (*Artocarpus heterophyllus*, Lam.) contains various secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids and has the potential for antibacterial activity in inhibiting wound healing, namely in *E. coli* and *S. aureus*. The strength of the antibacterial activity of the extract is included in the "strong" category.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

References

Chen, C-Y., Yin, H., Chen, X., Chen, T-H., Liu, H-M., Rao, S-S., Tan, Y-J., Qian, Y-X., Liu, Y-W., Hu, X-K., Luo, M-J., Wang, Z-X., Liu, Z-Z., Cao, J., He, Z-H., Wu, B., Yue, T., Wang, Y-Y., Xia, K., Luo, Z-W., Wang, Y., Situ, W-Y., Liu, W-E., Tang, S-Y., & Xie, H. (2020). Ångstrom-scale silver particle–embedded carbomer gel promotes wound healing by inhibiting bacterial colonization and inflammation. *Science Advances*, 6(43), eaba0942. https://doi.org/10.1126/sciadv.aba0942

Gupta, A.K., Rather, M.A., Jha, A.K., Shashank, A., Singhal, S., Sharma, M., Pathak, U., Sharma, D., & Mastinu, A. (2020). Artocarpus lakoocha Roxb. and Artocarpus heterophyllus Lam. Flowers: New sources of bioactive compounds. *Plants*, 9(10), 1-16. https://doi.org/10.3390/plants9101329

Gurning, K. (2020). Determination antioxidant activities methanol extracts of bangun-bangun (Coleus amboinicus L.) Leaves with DPPH method. Jurnal Pendidikan Kimia (JPKIM), 12(2), 62-69. https://doi.org/10.24114/jpkim.v12i2.19397

Gurning, K., Situmorang, R.F.R., Sinaga, E.T., Singarimbun, N., Sinaga, S.P., & Silaban, S. (2022). Determination of tannins content and antibacteria activity test of ethanol extract of sirih merah (*Piper Crocatum* Ruiz & pav.) leaf from North Sumatera Province Indonesia. *Oriental Journal of Chemistry*, 38(4), 967-971. https://doi.org/10.13005/ojc/380418

Juwitaningsih, T., Roza, D., Silaban, S., Hermawati, E., & Windayani, N. (2022). Phytochemical screening, antibacterial, antioxidant, and anticancer activity of Coffee parasite acetone extract (Loranthus ferrugineus Roxb). *Pharmacia*, 69(4), 1041-1046. https://doi.org/10.3897/pharmacia.69.e91427

Kuspradini, H., Putri, A.S., Egra, S., & Yanti. (2019). Short communication: In vitro antibacterial activity of essential oils from twelve aromatic plants fromEast Kalimantan, Indonesia. *Biodiversitas*, 20(7), 2039-2042. https://doi.org/10.13057/biodiv/d200733

Laguliga, J., Erviani, A., & Soekendarsi, E. (2021). Uji potensi getah jarak pagar Jatropha curcas Linn. terhadap kecepatan penyembuhan lluka bakar pada kulit tikus Rattus novergicus. *BIOMA: Jurnal Biologi Makasar*, 6(2), 74-83.

Nasri, N., Kaban, E., Gurning, K., Syaputra, H.D., & Satria, D. (2022). Aktivitas antibakteri ekstrak etanol daun pepaya (Carica papaya Linn.) terhadap bakteri Pseudomonas aeruginosa. *INSOLOGI: Jurnal Sains dan Teknologi*, 1(3), 252-259. https://doi.org/10.55123/insologi.v1i3.438

Ranasinghe, R.A.S.N., Maduwanthi, S.D.T., & Marapana, R.A.U.J. (2019). Nutritional and health benefits of jackfruit (Artocarpus heterophyllus lam.): a review. *International Journal of Food Science*, 2019, 4327183. https://doi.org/10.1155/2019/4327183

Sayogo, W., Widodo, A., & Dachlan, Y. (2017). Potensi +Dalethyne terhadap epitelisasi luka pada kulit tikus yang diinfeksi bakteri MRSA. *Jurnal Biosains Pascasarjana*, 19(1), 68-84.

Schneider, L., Korber, A., Grable, S., & Dissemond, J. (2007). InXuence of pH on wound-healing: A new perspective for wound-therapy?. *Arch Dematol Res*, 298, 423-420. https://doi.org/10.1007/s00403-006-0713-x

Silaban, S., Nainggolan, B., Simorangkir, M. Z., Pakpahan, P., & Gurning, K. (2022). Antibacterial activities test and brine shrimp lethality test of Simargaolgaol (Aglaonema modestum Schott ex Engl.) leaves from North Sumatera, Indonesia. *Rasayan Journal of Chemistry*, 15(2), 745-750. https://doi.org/10.31788/RJC.2022.1526911

Situmorang, R.F.R., Gurning, K., Kaban, V.E., Butar-Butar, J., & Perangin-Angin, S.A.B. (2022). Determination of total phenolic content, analysis of bioactive compound components, and antioxidant activity of ethyl acetate seri (Muntingia calabura L.) leaves from North Sumatera province, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, 10(A), 240-244. https://doi.org/10.3889/oamjms.2022.8362

Sumilat, D. (2019). Skrining aktivitas antibakteri beberapa jenis spons terhadap pertumbuhan strain bakteri Staphylococcus aureus, Escherichia coli, Staphylococcus saprophyticus, dan Pseudomonas aeruginosa. *Jurnal Ilmiah Platax*, 7(2), 455-461.

Tambunan, I.Y., Siringo-Ringo, E., Butar-Butar, M.J., & Gurning, K. (2023). GC-MS analysis of bioactive compounds and antibacterial activity of nangka leaves (Artocarpus heterophyllus Lam). *Pharmacia*, 70(1), 67-72. https://doi.org/10.3897/pharmacia.70.e97990

Thapa, R.K., Diep, D.B., & Tønnesen, H.H. (2020). Topical antimicrobial peptide formulations for wound healing: Current developments and future prospects. *Acta Biomaterialia*, 103, 52-67. https://doi.org/10.1016/j.actbio.2019.12.025

Zhou, J., Yao, D., Qian, Z., Hou, S., Li, L., Jenkins, A.T.A., & Fan, Y. (2018). Bacteria-responsive intelligent wound dressing: Simultaneous In situ detection and inhibition of bacterial infection for accelerated wound healing. *Biomaterials*, 161(1), 11-23. https://doi.org/10.1016/j.biomaterials.2018.01.024