

DOI: <https://dx.doi.org/10.18203/2319-2003.ijbcp20241279>

## Original Research Article

# Antibacterial activity of Kemloko fruit ethanol extract (*Phyllanthus emblica* L.) against *Staphylococcus aureus* and MRSA bacteria

Lidia Klorida Br Barus<sup>1</sup>, Masfria<sup>2,3\*</sup>, Denny Satria<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

<sup>3</sup>Nanomedicine Center of Innovation, Universitas Sumatera Utara, Medan, Indonesia

**Received:** 09 April 2024

**Revised:** 22 April 2024

**Accepted:** 24 April 2024

### \*Correspondence:

Dr. Masfria,

Email: [masfria@usu.ac.id](mailto:masfria@usu.ac.id)

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Indonesia is a tropical country, so the prevalence of infectious diseases caused by bacteria remains high. On the other hand, the high use of antibacterial ingredients in Indonesia causes bacterial resistance to existing antibacterial drugs. One of the herbal plants that some people widely use is Kemloko fruit. This study aimed to determine the activity of Kemloko fruit (*Phyllanthus emblica* L.) ethanol extract against the growth of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria.

**Methods:** Simplicia of Kemloko fruit were tested for secondary metabolites and characterization test of the simplicia. The antibacterial activity test was carried out using the diffusion method, where the disc paper was immersed in the test solution with the ratio of the test concentration.

**Results:** The results of phytochemical screening showed that the secondary metabolite compounds of Kemloko fruit are alkaloids, flavonoids, steroids, tannins, saponins, and glycosides. Antibacterial activity of ethanol extract of Kemloko fruit with agar diffusion method effectively inhibits *S. aureus* bacteria, namely at a concentration of 80 mg/ml, the inhibition zone is 14.1 mm, and at a concentration of 100 mg/ml, MRSA bacteria obtained an inhibition zone of 14.3 mm.

**Conclusions:** The ethanol extract inhibits the growth of *S. aureus* and MRSA.

**Keywords:** Kemloko fruit, Phytochemical screening, Characterization, Antibacterial, *Staphylococcus aureus*, MRSA

## INTRODUCTION

Infectious diseases are diseases that currently still require severe treatment. Bacteria are one of the biological agents that can cause infection.<sup>1</sup> Based on the national health profile; it is known that the incidence rate of infectious diseases is still relatively high in Indonesia. Bacteria that develop in the human body can cause several infections that must be addressed immediately so as not to develop into a more severe disease, one of which is by administering an antibiotic to inhibit the growth of

pathogenic microorganisms in the human body.<sup>2</sup> Biodiversity in Indonesia is abundant, especially in plants with properties similar to traditional medicines. Indonesian people utilize traditional medicinal plants as ingredients for the treatment of various diseases.<sup>3</sup> Medicinal plants are a valuable gift of nature, which can serve as a source of food and medicine for humans.<sup>4</sup>

The utilization of herbs for health maintenance and disease disorders is currently needed and needs to be developed, especially with soaring medical costs.<sup>5</sup> Various studies

have reported that Kemloko fruit (*Phyllanthus emblica* L.) is a good antioxidant. The obtained antioxidant ethanol extract of Kemloko fruit is more potent with an IC<sub>50</sub> value of 1.12 µg/ml than vitamin C with an IC<sub>50</sub> value of 4.44 µg/ml.<sup>6</sup> Methanol extracts of Kemloko fruit from some selected regions showed more potent antioxidant activity than commercial compounds and may be considered a potential source of plant antioxidants.<sup>7</sup> Phenolic compounds such as gallic acid are one of the main components of Kemloko fruit. Gallic acid is a natural polyphenolic compound that can be anti-bacterial against various pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.<sup>8</sup> Based on the results of the study of Kemloko fruit nanoparticle suspension has anti-bacterial activity of *S. aureus* with an effective concentration at a concentration of 100 mg/ml inhibition zone diameter of 14.23 mm and the *E. coli* with inhibition zone diameter of 14.06 mm.<sup>9</sup> The results of silver nanoparticle research with a concentration of 10 µg on *K. pneumoniae* bacteria showed good inhibition.<sup>10</sup> The aim of this research was to determine the activity of Kemloko fruit ethanol extract against the growth of *S. aureus* and the MRSA bacteria.

## METHODS

Research regarding testing the activity of Kemloko fruit ethanol extract was carried out in the period June-August 2023 at the microbiology laboratory, faculty of pharmacy, universitas Sumatera Utara, Medan, Indonesia. The sample used was Kemloko fruit (*Phyllanthus emblica* L.) obtained from Gunung Tua, Padang Lawas, Sumatera Utara.

### Material

The materials used in this study were Kemloko fruit, hydrochloric acid (HCl) reagent, ammonia, chloroform, magnesium, distilled water, sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), Mayer reagent, Dragendorff, Bouchardat, FeCl<sub>3</sub>10%, DMSO, Mueller Hinton agar (Merck), bacterial suspension, 70% ethanol, plastic wrap, aluminium foil, and parchment paper.

### Preparation of Kemloko fruit simplicia powder

Kemloko fruit is cleaned of impurities by washing thoroughly under running water and draining. Then, it was dried in a drying cabinet at 40-50°C. Next, the sample was mashed and stored in a plastic container in a protected place.

### Preparation of ethanol extract from Kemloko fruit

Preparation of ethanol extract of Kemloko fruit using maceration method with a ratio of 1:10. Kemloko fruit simplicia powder was put into a glass container, and ethanol solvent was added. Soak for 5 days while occasionally stirring, then allow to stand. Then filtered.

The process was repeated for 2 days; then, the entire macerate was collected and evaporated with a rotary evaporator until a thick extract was obtained.<sup>11</sup>

### Characterization of Kemloko fruit

Characterization of Kemloko fruit simplicia includes examining water content, water-soluble juice content, ethanol-soluble juice content, total ash content, and acid-insoluble ash content.<sup>12</sup>

### Phytochemical screening

Simplicia and ethanol extracts of Kemloko fruit were subjected to phytochemical screening, which included steroid/triterpenoid analysis, alkaloid analysis, glycoside analysis, saponin analysis, flavonoid analysis, and tannin analysis.

### Antimicrobial analysis

Mueller-Hinton agar media and antimicrobial preparations were dissolved in distilled water. Sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the media is cooled to a temperature of 45°C in a water bath. Then, 20 ml of media was poured into a sterile petri dish and allowed to solidify. Each petri dish containing MHA was levelled and allowed to solidify. Then, enter the concentration of ethanol extract of Kemloko fruit, positive control, and negative control using a micropipette and place it on the disc paper. Disc paper is inserted into the petri dish and allowed to stand for a while so that the diffusion process can occur. Next, the petri dish was incubated at 37°C for 24 hours. After incubation, calculate the diameter of the inhibition zone with a digital calliper. This was done three times. This was done to ensure that all inhibition zones in each experiment were obtained under the same experimental conditions.

### Observation and measurement of inhibition by agar diffusion method

Observations were made after 18-24 hours of incubation in an incubator. The area around the disk appears to have microbial sensitivity to the antimicrobial material used as the test material, expressed by the diameter of the inhibition zone in the clear zone formed. The diameter of the inhibition zone was measured in millimetres (mm) using a calliper.

## RESULTS

### Characterization of Kemloko fruit simplicia

The results of testing the characteristics of Kemloko fruit simplicia, which include examination of water content, water-soluble juice content, ethanol-soluble juice content, total ash content, and acid-insoluble ash content, are shown in Table 1.

**Table 1: Examination of simplicia powder characteristics.**

| Characterization of simplicia | Yield (%) |
|-------------------------------|-----------|
| Water content                 | 3.99      |
| Water soluble essence content | 33.54     |
| Ethanol-soluble juice content | 28.11     |
| Total ash content             | 7.32      |
| Acid insoluble ash content    | 0.93      |

Determination of water content is carried out to determine the water content in simplicia. Determination of water-soluble essence content to determine the level of polar chemical compounds contained in Kemloko fruit simplicia obtained results of 33.54%. Determination of ethanol soluble residual content to determine the concentration of soluble extracts in both polar and non-polar compounds in ethanol solvents obtained a result of 28.11%. Total ash content was determined to determine the level of inorganic substances contained in the simplicia of 7.32%, while the determination of acid insoluble ash content of 0.93% to determine level of inorganic substances insoluble in acid.

#### Phytochemical screening of Kemloko fruit ethanol extract

The content of secondary metabolites in the ethanol extract of Kemloko fruit has been tested qualitatively, showing the content of secondary metabolites, including alkaloids, flavonoids, steroids, saponins, tannins, and glycosides. The test results can be seen in Table 2 below.

**Table 2: Phytochemical screening results of Kemloko fruit.**

| Compound               | Results |
|------------------------|---------|
| Alkaloids              | +       |
| Flavonoids             | +       |
| Glycosides             | +       |
| Saponins               | +       |
| Tannins                | +       |
| Steroids/triterpenoids | +       |

(+): Contains a class of compounds and (-): Does not contain the compound class.

These results were identified based on the findings of the chemical analysis of secondary metabolites. This group was identified by forming a white precipitate on Meyer's reagent, a reddish-brown precipitate on Wagner's reagent, and Dragendorff. The three reagents showed positive results, whereas alkaloids are positive when 2 or 3 reagents show positive results. Examination using  $\text{FeCl}_3$  on an ethanol extract of Kemloko fruit showed positive results, namely a color change to a solid green, red, purple, and black color. This indicates that the ethanol extract of Kemloko fruit is positive for tannin compounds. Saponin examination by shaking with hot water and adding HCl 2N showed stable foam formation, so the ethanol extract of Kemloko fruit also contains saponin compounds.

Flavonoid examination showed positive results, also marked by the color change that occurs, which is reddish black, so it is favorable to contain flavonoids. The reaction using Lieberman-Bouchard reagent gives a red-purple color (indicating positive triterpenoids), while it shows a green-blue color (positive triterpenoids). The sample showed positive results on steroids and triterpenoids.<sup>13</sup>

#### Antibacterial activity of ethanol extract of Kemloko fruit

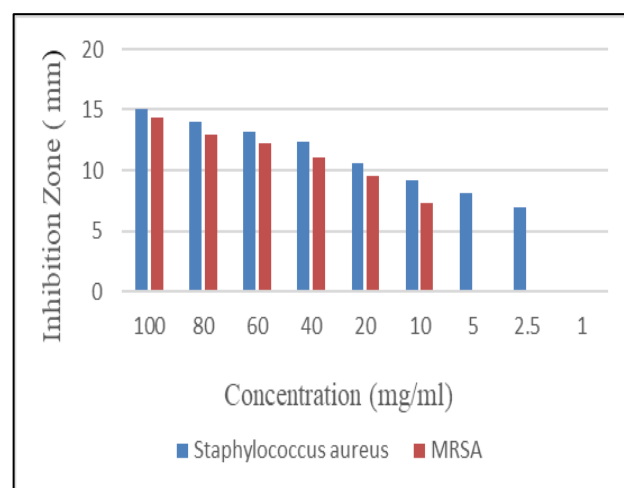
The results of the activity test of ethanol extract of Kemloko fruit against *S. aureus* and MRSA bacteria with test concentrations of 1, 2.5, 5, 10, 20, 40, 60, 80, and 100% for measurement of inhibition zones in the form of clear zones produced can be seen in Table 3.

**Table 3. Antibacterial activity results of ethanol extract of kemloko fruit.**

| Concentration (mg/ml) | Diameter of bacterial growth inhibition area (mm) |      |
|-----------------------|---------------------------------------------------|------|
|                       | <i>S. aureus</i>                                  | MRSA |
| 100                   | 15.0                                              | 14.3 |
| 80                    | 14.1                                              | 13.0 |
| 60                    | 13.4                                              | 12.2 |
| 40                    | 12.5                                              | 11.1 |
| 20                    | 10.3                                              | 9.5  |
| 10                    | 8.6                                               | 7.2  |
| 5                     | 8.0                                               | 6.3  |
| 2.5                   | 7.0                                               | -    |
| 1                     | -                                                 | -    |

(-): Does not inhibit.

Table 3 shows the antibacterial activity of Kemloko fruit extract against *S. aureus* and MRSA bacteria using the agar diffusion. DMSO was used as a solvent. Figure 1 illustrates the impact of different concentrations of Kemloko fruit ethanol extract.

**Figure 1: Effect of concentration of Kemloko fruit ethanol extract on the inhibition of *S. aureus* and MRSA aureus bacteria.**

## DISCUSSION

### *Characteristic testing of Kemloko fruit (Phyllanthus emblica L.) simplicial*

Kemloko fruit simplicial powder results meaning that the standardization of simplicial meets the requirements.<sup>14</sup> The high-water content that exceeds 10% can be an excellent medium to accelerate the growth of bacteria and fungi so that the quality of the simplicial decreases.<sup>15</sup> The determination of water-soluble juice content to determine the levels of polar chemical compounds such as flavonoids, glycosides, and saponins contained in Kemloko fruit simplicial. The result of the determination of ethanol soluble juice content to determine the content of juice that dissolves in ethanol solvents, both polar and non-polar compounds. Total and acid-insoluble ash content is determined by examining the mineral content of the extract, which can come from inorganic compounds.<sup>16</sup>

### *Phytochemical screening of Kemloko fruit (Phyllanthus emblica L.)*

The content of secondary metabolites in the ethanol extract of Kemloko fruit has been tested qualitatively, showing the content of secondary metabolites, including alkaloids, flavonoids, steroids, saponins, tannins, and glycosides. This result is consistent with prior research findings.<sup>17</sup> The presence of secondary metabolites is an important factor in bacterial inhibition.

### *Antibacterial activity test results of Kemloko fruit ethanol extract against S. aureus and MRSA bacteria*

The results show that the greater the concentration of the test, the greater the diameter of the inhibition zone. This is due to differences in the number of secondary metabolites in each concentration. The test results showed that the ethanol extract of Kemloko fruit more strongly inhibited *S. aureus* bacteria, namely at a concentration of 80 mg/ml, a zone of 14.1 mm was obtained.

Meanwhile, at a concentration of 100 mg/ml, the MRSA bacteria obtained an inhibition zone of 14.3 mm. *S. aureus* bacteria have polar peptidoglycan layer, so the flavonoids contained in the extract can penetrate the peptidoglycan layer more than the lipid layer, which is non-polar. Alkaloids are thought to have antibacterial activity by interfering with the constituent components of peptidoglycan in bacterial cells so that cells are not well formed and will cause cell death.<sup>18</sup> Saponins can bind to cytoplasmic membrane, disrupting and destabilising the cell membrane. Damage to the membrane will result in inhibited cell growth and even bacterial cell death.<sup>19</sup> Other metabolite compounds like tannins can also inhibit bacterial growth by coagulating protoplasm.<sup>20</sup>

However, the components responsible for the antibacterial activity of ethanol extract need to be clarified. Therefore, it will be worthwhile to isolate the active fractions of

Kemloko fruit for further testing to prepare an excellent antimicrobial agent against *S. aureus* and MRSA.

## CONCLUSION

Ethanol extract of Kemloko fruit contains secondary metabolite compounds of alkaloid, flavonoid, tannin, saponin, glycoside, and steroid/triterpenoid groups and can inhibit the growth of *S. aureus* and MRSA bacteria.

## ACKNOWLEDGEMENTS

Author would like to thank to department of pharmaceutical biology, faculty of pharmacy, universitas Sumatera Utara.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

1. Departemen Kesehatan RI. Profil Kesehatan Indonesia. Jakarta: Kementrian Kesehatan RI. 2019.
2. Widyatamaka SQ, Hilmi IL, Salman S. Article Review: Relationship Between Knowledge Level and Rational Antibiotic Use Behavior in Society. J Pharmaceut Sci. 2023;6(1):139-45.
3. Kusmana C, Hikmat A. Keanekaragaman Hayati Flora di Indonesia. J Natural Res Environmental Management. 2015;5(2):187-98.
4. Ahmad B, Hafeez N, Rauf A, Bashir S, Linfang H, Rehman MU, et al. *Phyllanthus emblica*: A comprehensive review of its therapeutic benefits. S Afr J Botany. 2021;138:278-310.
5. Vera Y, Yanti S. Penyuluhan Pemanfaatan Tanaman Obat Dan Obat Tradisional Indonesia Untuk Pencegahan Dan Penanggulangan Penyakit Hipertensi Di Desa Salam Bue. J Education Development. 2020;8(1):11.
6. Siregar S. Uji Aktivitas Antioksidan Ekstrak Etanol Buah Kemloko (*Phyllanthus emblica* L.) Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil). Universitas Sumatera Utara. Medan. 2019.
7. Liu X, Zhao M, Wang J, Yang B, Jiang Y. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. J Food Composition Analysis. 2008;21(3):219-28.
8. Sheoran S, Nidhi P, Kumar V, Gajender S, Uma RL, Anuradha S, et al. Altitudinal variation in gallic acid content in fruits of *Phyllanthus emblica* L. and its correlation with antioxidant and antimicrobial activity. Vegetos. 2019;32(2):387-96.
9. Silvia S. Formulasi dan Uji Aktivitas Suspensi Nanopartikel Buah Kemloko (*Phyllanthus Emblica* L.) terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli* (Doctoral dissertation, Universitas Sumatera Utara). 2022.

10. Renuka R, Devi KR, Sivakami M, Thilagavathi T, Uthrakumar R, Kaviyarasu K. Biosynthesis of silver nanoparticles using *Phyllanthus emblica* fruit extract for antimicrobial application. Biocatalysis Agricultural Biotechnol. 2020;24:101567.
11. Depkes RI. Farmakope Herbal Indonesia. Edisi II. Jakarta: Departemen Kesehatan RI. Halaman. 2017;311.
12. Depkes. Kebijakan Obat Tradisional Nasional. Jakarta: Direktorat Jenderal Bina Kefarmasian Dan Alat Kesehatan. Halaman. 2007;194-7.
13. Fansworth NR. Biological and Phytochemical Screening of Plants. J Pharmaceut Sci. 1996;55(3):263.
14. Kemenkes RI. Farmakope Herbal Indonesia. Edisi I. Jakarta: Kementerian Kesehatan RI. 2011.
15. Ditjen POM. Parameter Standar Umum Ekstrak Tumbuhan Obat. Cetakan Pertama. Jakarta: Departemen Kesehatan RI. Hal. 2000;10-32.
16. Depkes RI. Materia Medika Indonesia. Jilid VI. Jakarta: Departemen Kesehatan RI. Hal. 1995;300-6.
17. Singh E, Sharma S, Pareek A, Dwivedi J, Yadav S, Sharma S. Phytochemistry, traditional uses and cancer chemopreventive activity of Amla (*Phyllanthus emblica*): The Sustainer. J Appl Pharmaceut Sci. 2012;2(1):176-83.
18. Misrahanum M, Safarah Z, Ismail YS. Antibacterial activity of Mexican sunflower leaf *Tithonia diversifolia* (Hemsl.) A. Gray Aqueous extract against methicillin-resistant *Staphylococcus aureus*. Pharmacia. 2022;12(1):128-35.
19. Dong S, Yang X, Zhao L, Zhang F, Hou Z, Xue P. Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. Industrial Crops Products. 2020;149:112350.
20. Simangunsong T, Gaol EFL, Sianturi S. Effectiveness of bakung extract (*Crinum asiaticum* L.) as an analgesic in acetic acid-induced mice. J Farm Sains Dan Prakt. 2021;7(2):126-34.

**Cite this article as:** Barus LKB, Masfria, Satria D. Antibacterial activity of Kemloko fruit ethanol extract (*Phyllanthus emblica* L.) against *Staphylococcus aureus* and MRSA bacteria. Int J Basic Clin Pharmacol 2024;13:439-43.