Phytochemical Screening and Antimicrobial Activity of Matoa (*Pometia pinnata*) Leaves Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Matoa (*Pometia pinnata*) is a plant commonly used by the Indonesian people for traditional medicine due to its rich content of metabolites. This study aimed to analyze the phytochemical composition and antibacterial activity of matoa leaves The leaves were extracted using a 70% ethanol solvent with a ratio of 1:4 using the maceration method, and phytochemical screening was performed on the leaf extract. The antibacterial activity was tested against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The results indicated that the matoa leaf extract contains various metabolites, including triterpenoids, steroids, flavonoids, phenolics, saponins, tannins, and alkaloids. Secondary metabolite compounds in matoa leaves are thought to be able to inhibit microbial growth so that inhibition zones can be formed. Furthermore, the antibacterial activity demonstrated that the matoa leaf extract effectively inhibited the growth of *S. aureus* and *E. coli*. the largest inhibition zone is of 18.22 mm at 10% extract concentration, and *E. coli*, with an inhibition zone largest is of 10.87 mm at 10% extract concentration. While the smallest inhibition zone in *S. aureus* is 15.29 mm at 7.5% concentration, and in *E. coli* is 5% at 5% extract concentration.. This study suggests that matoa leaves have the potential to serve as natural bioactive compounds and antimicrobial agents in the clinical industry.

Keywords: Matoa, Phytochemical, Antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Matoa (*Pometia pinnata*) is a plant that has potential for health and commonly found in different regions of Indonesia. Matoa is a plant belonging to the Sapindaceae family and is widely used by communities as traditional medicine (Kurnianto et *al*., 2021). Almost all parts of the matoa plant, including the leaves, bark, fruit, and roots, can serve as traditional medicinal ingredients. The leaves of the matoa plant can be used to treat various ailments such as fever, skin pain, swelling from sprains, and even hypertension (Damayanti *et al*., 2023). Matoa leaves contain a variety of phytochemicals, including alkaloids, flavonoids, tannins, saponins, and steroids, which exhibit antibacterial (Risna, 2023). *Staphylococcus aureus* and *Escherichia coli* bacteria are examples of bacteria that represent gram positive and negative. These bacteria also cause health problems for humans.

Staphylococcus aureus and *Escherichia coli* are pathogenic bacteria that can cause infections in humans. *S. aureus* is a gram-positive bacteria associated with a range of diseases, including skin infections (such as boils and impetigo), pneumonia, sepsis, endocarditis, and food poisoning due to the production of enterotoxins (Frickmann *et al*., 2019). *E. coli*, on the other hand, is a type of gram-negative bacteria commonly found in the human gut. These bacteria can spread through contaminated food or water, leading to digestive tract diseases including diarrhea (Setiyanto *et al*., 2024). Infections caused by *S. aureus* and *E. coli* are particularly challenging due to their ability to resist antibiotics, making treatment difficult.

Increasing bacterial resistance to synthetic antibiotics creates an urgent need to find effective and safe treatment alternatives. Recent studies have investigated the antibacterial potential of ethanol extracts from matoa leaves against pathogenic bacteria. Matoa seed extract and the water fraction at a concentration of 500 mg/L were effective in inhibiting the growth of *E. coli* (Karnelasatri *et al*., 2024). In other study, ethanol extracts of matoa leaves at concentrations of 1%, 1.5%, and 2 can produced inhibition zones measuring 12.88 mm, 13.15 mm, and 11.36 mm against *S. aureus*, respectively, and 9.5 mm, 10.23 mm, and 12.1 mm against *E. coli* (Risna, 2023). Therefore, this study aims to determine the phytochemical content of matoa leaf extract and its potential in inhibiting the growth of *S. aureus* and *E. coli* at various concentrations.

RESEARCH METHODS

Preparation of Plant Materials

Matoa leaves are collected, then washed and cut into small pieces, and the total wet weight of the leaves was measured at 4000 grams. Subsequently, the leaf samples were dried in an oven at 50°C for 3x24 hours until the moisture content reached approximately 10%. After drying, the samples were blended into a fine powder and then sieved to obtain a uniform particle size (simplisia). The final weight obtained after drying and grinding was 1510 grams.

Matoa Leaf Extraction

Matoa leaf extraction was performed using the maceration method (Halifah *et al*., 2019). First, 250 grams of the mashed sample were weighed and then macerated with 70% ethanol solvent as much as 1000 ml or with a ratio of 1:4. The mixture was homogenized and stored for 24 hours. This maceration process was repeated three times. Afterward, the samples were filtered and combined. The resulting filtrate was then evaporated using a rotary evaporator for several hours with the aim of reducing the solvent content. The evaporated extract is placed in an oven at 40°C for several days with the aim of further reducing the solvent so that the extract becomes thick. Hasnaeni *et al*. (2019), the yield of the extract was calculated using the following formula:

$$\% \textbf{ of yield} = \frac{\textbf{Weigt of extract}}{\textbf{dry weigt of simplicia}} \times \mathbf{100}\%$$

Phytochemical Assay

Analysis of the phytochemical content of matoa leaves was carried out using the Harborne method (1987). The phytochemical content tested was triterpenoid, steroid, flavonoid, phenolic, and saponin. Sample testing was conducted at the Biology Laboratory of Makassar State University.

Antibacterial Activity

The antibacterial activity of matoa leaf extract was assessed using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. A suspension of bacteria (100 µL) was spread onto solid Mueller Hinton Agar (MHA) media. Discs made of paper were soaked with 20 µL of matoa leaf extract at varying concentrations of 5%, 7.5%, 10%, and 12.5%. Amoxicillin served as a positive control, while distilled water was used as a negative control. The samples were incubated for 24 hours at 37°C. After incubation, the ability of the matoa leaf extract to inhibit bacterial growth was evaluated by measuring the diameter of the inhibition zone using vernier calipers. All treatments were carried out three times.

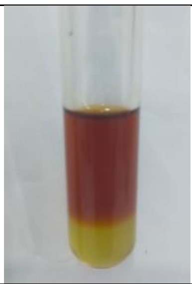
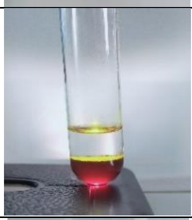
The data were analyzed using a variance analysis test (ANOVA) with SPSS 23 at a 95% confidence level. If the results were significantly different, the data were further tested using the Duncan test. The data obtained in the ANOVA test is in the form of a significance value that indicates whether or not there is an effect of the treatment given. If there is an effect indicated by a significance value of $\alpha < 0.05$, then it is continued with the Duncan test to analyze the difference in effect in each treatment that has a data form in the form of letter notation.

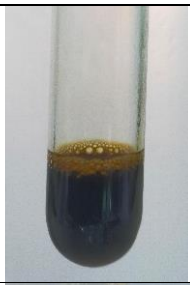
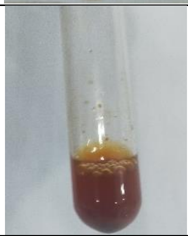
RESULTS AND DISCUCCION

Phytochemical Content

Matoa leaf samples were extracted using 70% ethanol. The extraction of 250 grams of dried matoa leaves yielded 48.51 grams of extract, resulting in a yield of 19.40%. Phytochemical screening revealed that matoa leaves contain triterpenoid, steroid, flavonoid, phenolic, saponins, tannin, and alkaloid. The results of the phytochemical tests are presented in Table 1 below.

Table 1. Phytochemical Test Results of Matoa Leaves (*Pometia pinnata*) with 70% Ethanol Solvent

No.	Phytochemical Test	Result	Positive Result	Description
1.	Triterpenoid		Formation of reddish color	Positive (+)
2.	Steroid		Formation of red ring	Positive (+)
3.	Flavonoid		Red or orange color forms	Positive (+)

4.	Phenolic		Blue-black color formed	Positive (+)
5.	Saponin		Forms a stable froth	Positive (+)
6.	Tannin		Forms a blue or green color and precipitate	Positive (+)
7.	Mayer’s Alkaloid		White precipitate forms	Positive (+)
8.	Wagner’s Alkaloid		Brown precipitate forms	Positive (+)
9.	Dragendroff’s Alkaloid		Orange precipitate forms	Negative (-)

Antibacterial Activity Against *Staphylococcus aureus*

The antibacterial activity of the matoa leaf extract against *Staphylococcus aureus* was tested using the 70% ethanol fraction at concentrations of 5%, 7.5%, 10%, and 12.5%. The results indicated that the extract successfully inhibited bacterial growth, as measured by the diameter of the inhibition zone (Table 2). Notably, a concentration of 10% of the matoa leaf extract produced the largest inhibition zone compared to the other tested concentrations.

Table 2. Antibacterial Activity of Matoa Leaf Extract Against Bacteria *S. aureus*

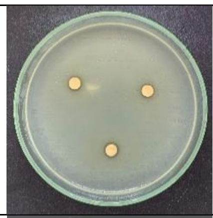
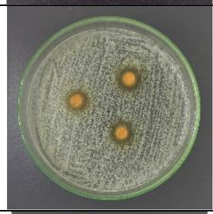
Concentration	Inhibition Zone Diameter (mm)			Average (mm)	Result
	1	2	3		
5%	17.47	16.52	17.33	17.11 ± 0.51^{c}	
7.5%	15.36	15.75	14.75	15.29 ± 0.50^{b}	
10%	18.66	17.98	17.72	18.12 ± 0.49^{d}	
12.5%	15.15	15.26	15.92	15.44 ± 0.42^{b}	
Positive Control (Amoxicillin 30ppm)	25.47	25.93	25.74	25.71 ± 0.23^{e}	
Negative Control (Aquades)	0	0	0	0 ± 0.00^{a}	

Numbers followed by the same letter in the same row are not significantly different in the Duncan's further test at the 5% level.

Antibacterial Activity Against *Escherichia coli*

Testing matoa leaf extract against E.Coli bacterial activity with 70% ethanol fraction at concentrations of 5%, 7.5%, 10% and 12.5% was able to inhibit bacterial growth based on the diameter of the inhibition zone (Table 3). The concentration of matoa leaf extract that has the best potential to inhibit bacterial growth is 7.5%, 10%, and 12.5%.

Table 3. Antibacterial Activity of Matoa Leaf Extract Against Bacteria *E. coli*

Concentration	Inhibition Zone Diameter (mm)			Average (mm)	Result
	1	2	3		
5%	8.74	8.43	7.89	8.35 ± 0.43[b]	
7.5%	10.638	10.738	11.138	10.84 ± 0.26[c]	
10%	10.518	11.078	11.028	10.87 ± 0.31[c]	
12.5%	10.268	10.508	10.483	10.42 ± 0.13[c]	
Positive Control (Amoxicillin 30ppm)	11.53	11.94	10.07	11,18 ± 0.98[c]	
Negative Control (Aquades)	0	0	0	0 ± 0.00[a]	

Numbers followed by the same letter in the same row are not significantly different in the Duncan's further test at the 5% level.

Phytochemical Content

Based on the results of phytochemical screening, matoa leaf extract contains secondary metabolites such as triterpenoid, steroid, flavonoid, phenolic, saponin, tannin and alkaloid that play role in antibacterial. Flavonoid can inhibit nucleic acid synthesis, compromise the function of the cytoplasmic membrane, and disrupt bacterial energy metabolism (Xie *et al*., 2015). Saponin, which can possess either triterpenoid or steroid scaffolds along with various carbohydrate structures, can lead to cell membrane lysis and inhibit the synthesis of bacterial biofilms (Li & Monje-Galvan, 2023). Additionally, alkaloid exhibit antibacterial activity by interfering with bacterial cell wall synthesis, altering cell membrane permeability, and inhibiting bacterial metabolism as well as the synthesis of nucleic acids and proteins (Casciaro *et al*., 2020). Tannin compounds also serve as antibacterial agents, inhibiting bacterial growth through mechanisms such as chelating iron, disrupting cell wall synthesis, damaging cell membranes, and hindering pathways for fatty acid biosynthesis in bacteria (Farha *et al*., 2020). The *Melastoma malabathricum* plant, which contains metabolites like phenols and flavonoids, has been shown to inhibit the growth of various bacterial species, including *S. epidermidis*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. aureus*, and *P. mirabilis* (Apridamayanti *et al*., 2021).

Antibacterial Activity

The 70% solvent has a polarity level that is closest to the polarity level of secondary metabolite compounds so that it can attract more secondary metabolite compounds contained in the leaves (Guna, *et al*., 2020). The use of 70% solvent functions to attract secondary metabolite compounds contained in the leaves which can act as antibacterials. Antibacterial activity tests demonstrate that matoa leaf extract and 70% ethanol fraction are effective at inhibiting the growth of *S. aureus and E. coli*. Risna (2023) found that ethanol extract of matoa leaves at concentrations of 1%, 1.5%, and 2% effectively inhibited the growth of *S. aureus* and *E. coli*, as measured by the size of the inhibition zones. Variations in the inhibition zones can be attributed to differences in the peptidoglycan layers of the bacterial cell walls (Sukmiwati *et al*., 2018).

Matoa leaf extract can inhibit the growth of *S. aureus* and *E. coli* bacteria. The largest inhibition zone formed in the growth of *S. aureus* bacteria is 18.12 mm at a concentration of 10% while the smallest inhibition zone is 15.29 mm at a concentration of 7.5%. In *E. coli* bacteria, the largest inhibition zone is 10.87 mm at a concentration of 10% while the smallest inhibition zone is 8.35 mm at a concentration of 5%. This growth inhibition is due to the synergistic effects of secondary metabolite compounds that damage bacterial cell membranes and inhibit bacterial activity (Islami *et al*., 2021; Pirdina *et al*., 2021).

The different diameters of the inhibition zones at each concentration are caused by several factors, namely differences in the levels of antibacterial compounds at each extract concentration, the ability of antibacterial compounds in the extract to diffuse into the media, and the sensitivity of bacteria to antibacterial compounds in the extract (Apriliantisyah, *et al*., 2022). In this test, the largest diameter of the inhibition zone was at a concentration of 10% for both bacteria. This is thought to be because the higher the concentration, the more antibacterial compounds will be contained so that the larger the inhibition zone that will be formed, but the higher the concentration will also reduce the solubility of the extract, thereby reducing the ability of antibacterial compounds to diffuse into the media which results in bacterial inhibition becoming less effective (Nurhamidin, *et al*., 2021). The two bacteria also showed differences in the diameter of the inhibition zone. In *S. aureus* bacteria, which are gram-positive, the largest diameter is 18.12 mm, while *E. coli*, which are gram-negative, have a diameter of 10.87 mm. This is due to differences in the cell wall structure of the two types of bacteria. The cell wall structure of gram-positive bacteria is simpler so it is more sensitive to antibacterial compounds, while the cell wall of gram-negative bacteria is more complex so it is more resistant to antibacterial compounds (Goetie, *et al*., 2022).

CONCLUSION

In conclusion, the findings show that matoa leaf extract contains various secondary metabolite compounds, including triterpenoids, steroids, flavonoids, phenolics, saponins, tannins, and alkaloids. Matoa leaf extract and 70% ethanol fraction both have antibacterial activity by effectively inhibiting the growth of *S. aureus*, with the largest inhibition zone of 18.22 mm at an extract concentration of 10%, and *E. coli* with the largest inhibition zone of 10.87 mm at an extract concentration of 10%. While the smallest inhibition zone in *S. aureus* is 15.29 mm at a concentration of 7.5%, and in *E. coli* is 5% at an extract concentration of 5%.%.

REFERENCE

Apridamayanti, P., Sari, R., Rachmaningtyas, A., Aranthi, V. (2021). Antioxidant, antibacterial activity and FICI (Fractional Inhibitory Concentration Index) of ethanolic extract of *Melastoma malabathricum* leaves with amoxicillin against pathogenic bacteria. *Nusantara Bioscience*, Vol. 13, No. 2, 140-147. https://doi.org/10.13057/nusbiosci/n130202

Apriliantisyah, W., Haidir, I., Sodiqah, Y., Said, M. F. M. (2022). Daya hambat ekstrak kunyit (Curcucma domestica Val) terhadap bakteri Staphylococcus aureus dan Escherichia coli. *Fakumi Medical Journal: Jurnal Mahasiswa Kedokteran*, Vol. 2, No. 10, 694-703. https://doi.org/10.33096/fmj.v2i10.127

Casciaro, B., Mangiardi, L., Cappiello, F., Romeo, I., Rosa, M., Iazzetti, A., Calcaterra, A., Goggiamani, A., Ghirga, F., Mangoni, M. L., Botta, B., Quaglio, D. (2020). Naturally-Occurring Alkaloids of Plant Origin as Potential Antimicrobials against. *Molecules*, Vol. 25, No. 16, 3619. https://doi.org/10.3390/molecules25163619

Damayanti, F., Malik, A., Dahlia, A. A. (2023). Skrining Fitokimia dan Penetapan Kadar Flavonoid Total Ekstrak Daun Matoa (*Pometia pinnata*) Menggunakan Metode Spektrofotometri UV-Vis. *Makassar Natural Product Journal*, Vol. 1, No. 4, 2023-2191.

Farha, A. K., Yang, Q. Q., Kim, G., Li, H. Bin, Zhu, F., Liu, H. Y., Gan, R. Y., Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, Vol. 38. https://doi.org/10.1016/j.fbio.2020.100751

Frickmann, H., Hahn, A., Berlec, S., Ulrich, J., Jansson, M., Schwarz, N. G., Warnke, P., Podbielski, A. (2019). On the Etiological Relevance of *Escherichia coli* and *Staphylococcus aureus* in Superficial and Deep Infections-A Hypothesis-Forming, Retrospective Assessment. *European Journal of Microbiology and Immunology*, Vol. 9, No. 4, 124-130. https://doi.org/10.1556/1886.2019.00021

Goetie, I. H., Sundu, R., Supriningrum, R. (2022). Uji aktivitas antibakteri ekstrak kulit batang sekilang (*Embelia borneensis* Scheff) terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus* menggunakan metode disc diffusion. *Jurnal Riset Kefarmasian Indonesia*, Vol. 4, No. 2, 144-155. https://doi.org/10.33759/jrki.v4i2.260

Guna, I. M. A. D., Putra, I. N. K., Wiadyani, A. A. I. S. (2020). Pengaruh konsentrasi etanol terhadap aktivitas antioksidan ekstrak daun Rambusa (*Passiflora foetida* L.) menggunakan metode ultrasonic assisted extraction (UAE). *Jurnal Itepa*, Vol. 9, No. 3, 291-300.

Halifah, P., Hartati, Rachmawaty, Yusminah, H., Roshanida, A. R. (2019).

Phytochemical screening and antimicrobial activity from *sonneratia caseolaris* fruit extract. *Materials Science Forum*, Vol. 967, 28-33. https://doi.org/10.4028/www.scientific.net/MSF.967.28

Harborne, J. B. (1998). *Phytochemical Methods A Guide To Modern Techniques of Plant Analysis (3th Edition).* Chapman and Hil. London.

Hasnaeni, Wisdawati, Usman, S. (2019). Pengaruh Metode Ekstraksi Terhadap Rendemen dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (*Lunasia amara* Blanco). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*, Vol. 5, No. 2, 175-182.

Islami, D., Anggraini, L., Wardaniati, I. (2021). Aktivitas Antioksidan dan Skrining Fitokimia dari Ekstrak Daun Matoa *Pometia pinnata*. *Jurnal Farmasi Higea*, Vol. 13, No. 1, 30. https://doi.org/10.52689/higea.v13i1.328

Karnelasatri, Yacob, I. A., Andareas, P., Santoso, F. R. C., Novia, J. (2024). Antibacterial Activity Assay on *Escherichia Coli* of The Matoa Seeds Active Fraction. *Jurnal Farmasi Galenika*, Vol. 10, No. 2, 234-243. https://doi.org/10.22487/j24428744.2024.v10.i2.16283

Kurnianto, E., Rahman, I. R., Farmasi, H. (2021). Skrining Fitokimia Ekstrak Etanol Daun Matoa Yang Berasal Dari Pontianak Timur Dengan Variasi Konsentrasi Pelarut. *Jurnal Komunitas Farmasi Nasional*, Vol. 1, No. 2, 131-138.

Li, J., and Monje-Galvan, V. (2023). In Vitro and In Silico Studies of Antimicrobial Saponins: A Review. *Processes*, Vol. 11, No. 10. https://doi.org/10.3390/pr11102856

Nurhamidin, A. P., Fatimawali, F., Antasionasti, I. (2021). Uji Aktivitas Antibakteri Ekstrak N-Heksan Biji Buah Langsat (*Lansium domesticum* Corr) Terhadap Bakteri Staphylococus Aureus Dan Klebsiella Pneumoniae. *Pharmacon*, Vol. 10, No. 1, 748-755. https://doi.org/10.35799/pha.10.2021.32772

Pirdina, M. W., Wijayanti, F., Permata Sari, S. (2021). Antibacterial Activity Test of Ethanol Extract of Matoa Leaf (*Pometia pinnata*) against *Salmonella typhi* Article History. *Sainstek: Jurnal Sains Dan Teknologi*, Vol. 13, No. 2.

Risna. (2023). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Matoa (*Pometia Pinnata* J. R & G.Forst.) terhadap Pertumbuhan *Staphylococcus Aureus* dan *Escherichia Coli*. *Jurnal Keperawatan Silampari*, Vol. 6, No. 2. https://doi.org/10.31539/jks.v6i2.4725

Setiyanto, R., Meitasari, A. D., Natasya, S. C., Sari, D. W. (2024). Antibacterial Potential of Dewandaru Leaves (*Eugenia uniflora* L.) Against *Escherichia coli*: In Vitro Study. *Sriwijaya Journal of Internal Medicine*, Vol. 2, No. 1, 77-83. https://doi.org/10.59345/sjim.v2i1.138

Sukmiwati, M., Diharmi, A., Mora, E., Susanti, E. (2018). Aktivitas Antimikroba Teripang Kasur (*Stichopus vastus* Sluiter) dari Perairan Natuna Kepulauan Riau. *Jurnal Pengolahan Hasil Perikanan Indonesia*, Vol. 21, No. 2, 328-335. https://doi.org/10.17844/jphpi.v21i2.23088

Xie, Y., Yang, W., Tang, F., Chen, X., Ren, L. (2015). Antibacterial Activities of Flavonoids : Structure-Activity Relationship and Antibacterial Activities of Flavonoids : Structure-Activity Relationship and Mechanism. *Current Medicinal Chemistry*, Vol. 22, 132-149. https://doi.org/10.2174/0929867321666140916113443