TEST THE EFFECTIVENESS OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES ON TURMERIC (CURCUMA LONGA LINN.)

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ABSTRACT

Research on Turmeric (Curcuma longa Linn.) is essential in evaluating antibacterial effects against Staphylococcus epidermidis, especially in biofilm formation. One interesting approach is the development of curcumin nanoparticles, as they can enhance antibacterial activity. The methods used in this study include turmeric collection and processing, simplistic making, ethanol extraction, as well as testing antioxidant activity with DPPH method (1,1-diphenyl-2-picrylhydrazil) and antibacterial activity with minimum inhibitory concentration method (KHM). The results showed that turmeric ethanol extract effectively inhibited the growth of Staphylococcus epidermidis, especially at concentrations of 300 mg / mL with an average inhibitory zone diameter reaching 10.12 mm. The negative control shows a consistent inhibitory zone at 6.00 mm. Antimicrobial compounds, particularly flavonoids in turmeric extract, damage protein structures and bacterial cell membranes, resulting in bacterial cell death. These results provide a deeper understanding of turmeric extract's potential as an effective antibacterial agent against Staphylococcus epidermidis. In addition, turmeric ethanol extract also showed significant antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis at various concentrations, with the largest inhibition zone at 300 mg/mL. Nonetheless, more research is needed to understand the mechanism of action and potential side effects in greater depth. Thus, turmeric has the potential to be an exciting alternative in the development of natural antibacterial agents that are effective and safe to use in clinical practice.

Keywords: - *Turmeric (Curcuma longa Linn.), Antibacterial, Staphylococcus epidermidis, Biofilm, Antibacterial activity*

1. BACKGROUND

Staphylococcus epidermidis, as a member of the genus Staphylococcus, has significant differences with Staphylococcus aureus because it is coagulase negative. This negative coagulase nature means that Staphylococcus epidermidis does not produce coagulase enzymes, distinguishing it from Staphylococcus aureus, which produces coagulase enzymes. The coagulase enzyme in Staphylococcus aureus plays a role in aiding blood clotting, thereby facilitating further infection and invasion in the body. As a negative coagulase, Staphylococcus epidermidis tends to be less virulent and more commonly found as part of the normal flora of human skin and mucous membranes (Lestari & Asri, 2021). However, despite its routine nature, Staphylococcus epidermidis can cause nosocomial infections, especially in patients with implanted medical devices. This condition is essential because these bacteria can form biofilms on the surface of prosthetic devices, making them difficult to treat and can cause serious infections, especially in patients who are very young, very old, or in immunocompromised states. Thus, the main difference between Staphylococcus epidermidis and Staphylococcus aureus lies in the coagulase properties, which provide essential implications in the virulence, spread, and ability of these bacteria to cause infection. As part of the normal flora of the skin and mucous membranes of humans, these bacteria can be found in significant numbers,

reaching 10 – 10^6 colony-forming units (CFU)/cm^2 in healthy human skin and mucous membranes. Although expected, Staphylococcus epidermidis can be a cause of nosocomial infections, especially in patients with implanted medical devices such as knee prosthesis, cerebrospinal fluid shunts, central venous catheters, and intravascular catheters, especially in patients who are very young, very old, or in immunocompromised states. Up to 75% of infections caused by coagulase-negative staphylococci, including Staphylococcus epidermidis, occur in implanted medical devices, with a high probability of bacteremia cases (Astari & Rialita, 2021).

Reported infections in patients with the use of pacemakers and urinary tract catheters led to significant therapeutic challenges. Staphylococcus epidermidis bacteria can form biofilms, often involved in infections in implanted medical devices. This biofilm is an extracellular layer that protects bacteria from antibiotics and the body's defenses, making treatment challenging. The increasing antibiotic resistance to Methicillin and Penicillin further complicates the problem. Research shows that some strains of Staphylococcus epidermidis have developed significant levels of antibiotic resistance, exacerbating the treatment situation of infections caused by these bacteria (Otto, 2009). Faced with this problem, a new therapeutic approach is needed. One promising approach is the exploration of natural materials. As a country with abundant biodiversity, Indonesia has great potential in discovering natural materials that can be used in traditional medicine. With about 20,000 medicinal plants, most have yet to be fully explored. About 1000 plants have been recorded, and 300 have been used as traditional medicine. Traditional medicine consists of ingredients or herbs derived from plants, animals, minerals, sarin preparations (galenic), or mixtures of these ingredients that are traditionally used for treatment and can be applied by the norms prevailing in society. Thus, further exploration and research on natural ingredients, especially medicinal plants, can be an essential step in overcoming the problem of Staphylococcus epidermidis infection, which is challenging to treat and resistant to antibiotics (Hamiyati & Laratmase, 2021).

Turmeric rhizomes (Curcuma longa) are one of the medicinal plants that can maintain health naturally in Indonesia. This plant optimally improves the body's work system, has antibacterial properties against the skin, and acts as an anti-inflammatory and antioxidant. Turmeric rhizomes contain alkaloids, flavonoids, tannins, curcumin, and essential oils. This content makes turmeric valuable as a traditional medicine for appetite enhancers, wound treatment, overcoming itching, antidiarrheal, and as an antibacterial. Turmeric rhizome extract with concentrations of 20%, 30%, and 40% inhibited the growth of Staphylococcus aureus bacteria with inhibitory zones of 17.67 mm, 20.67 mm, and 23 mm, respectively. These findings confirm turmeric's potential as a valuable resource in traditional medicine, particularly in fighting bacterial infections. Turmeric plants (Curcuma longa L.) have potential as natural antibiotic candidates, and this study was conducted to evaluate the antibacterial effect of turmeric ethanol extract against Staphylococcus epidermidis as a first step in overcoming the challenges of bacterial infections that are difficult to treat (Afriyana et al., 2023).

Research conducted by Pangemanan (2016) revealed that turmeric rhizome extract has antibacterial effects against bacteria Staphylococcus aureus and Pseudomonas sp., which can be attributed to the presence of curcumin active substances in turmeric rhizomes (Pangemanan et al., 2016). Curcumin, a polyphenolic compound that is hydrophobic, is present in turmeric and has been known to have various benefits, including as an antibacterial, antioxidant, anti-inflammatory, antiviral, antifungal, antiparasitic, antidiabetic, antiallergic, antiarthritis, hepatoprotective, neuroprotective, nephroprotective, and anti-HIV (El-Saadony et al., 2022). Other studies say curcumin is an anti-biofilm, increasing bacterial sensitivity to antibacterials. The essential oil in turmeric, containing sesquiterpene compounds and isoflavones, also has an antibacterial effect. Although curcumin has therapeutic benefits, its low solubility in water and bioavailability are challenges. Therefore, the development of nanoparticle-based drugs, especially silica nanoparticles, is carried out to improve the solubility and bioavailability of curcumin. Research shows that curcumin-sized nanoparticles are more soluble in water and have better antibacterial activity against various types of bacteria. Curcumin nanoparticles exhibit the ability to attach to bacterial cell walls, damage peptidoglycan, and penetrate into cells, causing damage to cell organelle structures and eventually cell death. This shows the potential of developing nanoparticle-based curcumin as a more effective antibacterial agent (Kali et al., 2016).

Based on the description above and some related literature, researchers are interested in researching antioxidant activity using the DPPH method and antibacterial methods using the Minimum Inhibition Concentration (KHM) method from turmeric (Curcuma longa Linn.).

2. RESEARCH METHODS

The experimental research conducted in January 2024 involves collecting, processing, and extracting Turmeric (Curcuma longa Linn.) to produce ethanol extract. Antioxidant activity testing was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazil) free radical restriction method, while antibacterial activity was tested by the minimum

inhibitory concentration method. Laboratory equipment such as blenders, drying cabinets, electric ovens, incubators, and UV/Vis spectrophotometers were used in this study. The materials used include peridot leaves (Saurauia vulcani), various solvents, bacterial growth media, and test bacteria Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228. The process of sampling, extraction, and testing is carried out carefully to obtain relevant information related to the antioxidant and antibacterial activity of turmeric, with the hope that the results of this research can contribute to the development of new therapies, especially related to the handling of bacterial infections and antioxidant properties of turmeric.

3. RESEARCH RESULTS

Phytochemical screening tests are carried out to determine and identify the components of bioactive compounds contained in Turmeric extract (Curcuma longa Linn.). Some active compound components identified include alkaloids, steroids/triterpenes, saponins, tannins, flavonoids, and glycosides. The screening results of Turmeric extract (Curcuma longa Linn.) extracted using ethyl acetate solvent can be seen in Table 1

Tabel 1. Hasil Uji Skrining Fitokimia Ekstrak Kunyit (Curcuma longa Linn.)

Bioactive Compounds	**Turmeric Extract (Curcuma longa Linn.)**
Alkaloids	+
Flavonoid	+
Saponins	+
Tannins	+
Streroid/Triterpenoid	-
Glikosida	+

Information:
(+) = contains compounds
(-) = contains no compounds

Based on the results of qualitative analysis of the active components of Turmeric extract extracted ethyl acetate solvent above, it shows that Turmeric extract (Curcuma longa Linn.) with ethyl acetate solvent contains almost all secondary metabolites, except steroids/triterpenoids. The results of phytochemical screening tests show that turmeric extract contains colloids, flavonoids, saponins, tannins, and glycosides, while it does not contain steroids/triterpenoids. The phytochemicals in turmeric have bioactive potential that can provide specific health benefits. Alkaloids have antibacterial abilities as well as pharmacological effects as analgesics and anesthetics. The mechanism of bacterial inhibition by this compound is thought to be by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death. Saponins have potential as antimicrobial compounds because they reduce the permeability of bacterial cell walls so that they can enter the bacterial cytosol and inhibit their growth. Another metabolite compound found in turmeric extract is tannins. Tannins are phenol compounds that cause damage to cell wall polypeptides; the mechanism of tannin inhibition occurs using bacterial walls that have been lysed due to saponin and flavonoid compounds, causing tannin compounds to enter bacterial cells and coagulate bacterial cell protoplasm quickly. Tannins act as antibacterial to inhibit reverse transcriptase enzymes and DNA topoisomerase so that bacterial cells cannot form.

Testing antioxidant activity on turmeric ethanol extract (Curcuma longa Linn.) using the DPPH method using UV-VIS spectrophotometer with wavelength 516 nm. The following are the results of the antioxidant activity test can be seen in Table 2. the following:

Table 2. Results of Turmeric Ethanol Extract Absorbance Measurement (Curcuma longa Linn.)

No	Concentration (ppm)	Absorbance	% Damping
1.	Blanko	0,778	0
2.	100 mg/mL	0,104	78,441

3.	50 mg/mL	0,114	74.448
4.	25 mg/mL	0,433	47,118
5.	12,5 mg/mL	0,464	31,301

The absorbance measurement results showed that the concentration of turmeric ethanol extract at 100 mg / mL had the lowest absorbance, which was 0.104, with a dampening percentage of 78.441%. Furthermore, absorbance increased with the decrease in turmeric extract concentration, with a concentration of 12.5 mg/mL having the highest absorbance of 0.464 but a lower dampening percentage of 31.301%. High absorbance indicates turmeric extract's capacity to absorb light, while the attenuation percentage indicates its effectiveness in reducing the intensity of light transmitted. The results of measuring the diameter of the clear zone of antibacterial activity of methanol extract of string bean leaves against *bacteria Staphylococcus aureus* and *Staphylococcus epidermidis* can be seen in Tables 3 and 4 below:

Table 3. Antibacterial Activity Test Results of Turmeric Ethanol Extract (Curcuma longa Linn.) *Stapylococcus aureus bacteria*

Concentration (mg/mL)	P1	P2	P3	X	THAT
300	11,3	11,0	11,0	10,38	0,13
200	9,1	9,3	9,3	9,41	0,15
100	8,6	8,3	8,1	8,41	0,13
50	7,8	8,4	7,4	7,35	0,11
25	7,3	6,3	6,4	6,33	0,13
12,5	6,3	6,3	6,4	6,34	0,11
Towards-	6	6	6	6,00	0,00

Table 4. Antibacterial Activity Test Results of Turmeric Ethanol Extract (Curcuma longa Linn.) *Stapylococcus epidermidis bacteria*

Concentration (mg/mL)	P1	P2	P3	X	THAT
300	13,1	13,2	13,6	10,12	0,14
200	9,1	9,6	9,2	8,34	0,32
100	8,2	8,1	8,1	8,32	0,31
50	7,2	7,1	8,3	7,26	0,52
25	6,1	6,1	6,7	6,44	0,34
12,5	6,9	6,1	6,2	6,23	0,05
6,25	6	6	6	6,00	0,00
Towards-	6	6	6	6,00	0,00

Table 3. shows the results of the antibacterial activity test of turmeric ethanol extract (Curcuma longa Linn.) against

Staphylococcus epidermidis bacteria at various concentrations. The diameter of the inhibitory zone (X) is measured from three repetitions (P1, P2, P3) for each concentration, with the mean value (X) and standard error mean (SEM) recorded. These results show that the concentration of 300 mg/mL shows the highest antibacterial activity with an average inhibitory zone diameter of 10.12 mm. Although there are slight variations between repetitions (P1, P2, P3) at each concentration, relatively small SEM values indicate the accuracy and consistency of the data. The negative control (K-) showed that without the extract treatment, the diameter of the inhibitory zone remained constant at 6.00 mm. These results provide additional evidence that turmeric ethanol extract has antibacterial potential, and the concentration of the extract plays a vital role in determining its effectiveness against Staphylococcus epidermidis.

Table 4. presents antibacterial activity test results of turmeric ethanol extract (Curcuma longa Linn.) against Staphylococcus epidermidis bacteria at various concentrations. The diameter of the inhibitory zone (X) is measured by three repetitions (P1, P2, P3) for each concentration, with the mean value (X) and standard error mean (SEM) recorded. Results showed that the higher the concentration of turmeric ethanol extract, the greater the diameter of its inhibition zone against Staphylococcus epidermidis. At a concentration of 300 mg/mL, the extract showed the highest antibacterial activity with an average inhibitory zone diameter of 10.12 mm. The negative control (K-) showed that without the extract treatment, the diameter of the inhibitory zone remained about 6.00 mm. These results indicate that turmeric ethanol extract has potential as an antibacterial agent, with its concentration playing a role in determining its effectiveness against Staphylococcus epidermidis.

Antimicrobial compounds can cause damage to the cell wall and damage to the cell membrane in the form of the denaturation of proteins and fats that make up the cell membrane. Turmeric ethanol extract (Curcuma longa Linn.) effectively inhibits the growth of gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermis*; this is likely due to antibacterial activity influenced by several factors, namely the concentration of the extract and the type of bacteria inhibited.

Based on the table obtained, pathogenic bacterial strains are used for antibacterial screening of Turmeric ethanol extract (Curcuma longa Linn.) from the maceration extraction process, namely *Staphylococcus* aureus and *Staphylococcus epidermidis.* Inhibits bacterial growth. Growth and number of bacteria < 10 colonies. KHM is the minimum concentration of antimicrobial substances that can inhibit bacterial growth after 24 hours of incubation. No known bacterial colony is growing, as observed by the number of bacterial colonies growing (Astari & Rialita, 2021). The diameter of the inhibitory zone of 5 mm or less is categorized as weak, the diameter of the inhibitory zone of 5-10 mm is classified as medium, the diameter of the inhibitory zone of 10-20 mm is categorized as vital, and the inhibitory zone of 20 mm or more is classified as very strong. This study shows that the higher the concentration of extract, the greater the amount of antibacterial compounds released, thus facilitating the penetration of these compounds into cells; in other words, the higher the concentration of extracts and the length of contact time, the more active antibacterial activity, it is stated that gram-positive bacteria whose outer membrane consists of more peptidoglycan layers than gram-negative whose outer membrane consists of lipopolysaccharides namely lipids, polysaccharides and proteins.

The cell wall of gram-negative bacteria contains much less peptidoglycan than gram-positive, so the permeability of gram-positive bacteria is lower than that of gram-negative bacteria. With low permeability, the active substance from the methanol extract of loning plant leaves will have difficulty penetrating the cell membrane of gram-positive bacteria so that the bacterial effect is less optimal on growing bacterial cells and causes cell death. Flavonoid compounds can form complexes with bacterial cell proteins through hydrogen bonds (Hafizah et al., 2015). The structure of the cell wall and membrane of the bacterial cytoplasm containing proteins becomes unstable because the protein structure of bacterial cells becomes damaged due to hydrogen bonds with flavonoids, causing bacterial cell proteins to lose their biological activity. As a result, the permeability function of bacterial cells is disrupted, and bacterial cells will undergo lysis, resulting in bacterial cell death (Hadrian Nugro Astuti et al., 2016).

Flavonoid compounds are thought to have a mechanism of action that denatures bacterial cell proteins and damages cell membranes irreparably (Fadhilah, 2019). Flavonoids are also lipophilic, which will damage microbial membranes because flavonoids contain phenol compounds. The growth of bacteria *Staphylococcus* aureus and *Staphylococcus epidermidis* can be disrupted due to phenol compounds, where this compound is an acidic alcohol. Besides, phenol also can denature proteins and damage cell membranes. Acidic conditions in the presence of phenols can affect the growth of *Staphylococcus* aureus and *Staphylococcus epidermidis* bacteria (Yuliati, 2017).

4. CONCLUSION

Based on the results of studies that have been carried out, it can be concluded that e turmeric ethanol extract (Curcuma longa Linn.) shows significant antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis at various concentrations, with the largest zone of inhibition at a concentration of 300 mg / mL. These

results demonstrate turmeric's potential as a source of natural antibacterial agents that can be used to develop health products. However, more research is needed to understand its mechanism of action and potential side effects. Turmeric ethanol extract (Curcuma longa Linn.) has potential as an exciting alternative in developing natural antibacterial agents.

6. REFERENCES

Afriyana, R., Junando, M., & Nurmasuri. (2023). Potensial Ekstrak Herbal Kunyit (Curcuma Longa) Sebagai Anti Bakteri dan Anti Inflamasi. *Agromedicine*, *10*(1), 128–132.

Astari, S. M., & Rialita, A. (2021). *Aktivitas Antibakteri Isolat Bakteri Endofit Tanaman Kunyit (Curcuma longa L .) Terhadap Pertumbuhan Staphylococcus aureus*. *8*(2), 9–16. https://doi.org/10.33096/jffi.v8i2.644

El-Saadony, M. T., Yang, T., Korma, S. A., Sitohy, M., Abd El-Mageed, T. A., Selim, S., Al Jaouni, S. K., Salem, H. M., Mahmmod, Y., Soliman, S. M., Mo'men, S. A. A., Mosa, W. F. A., El-Wafai, N. A., Abou-Aly, H. E., Sitohy, B., Abd El-Hack, M. E., El-Tarabily, K. A., & Saad, A. M. (2022). Impacts of Turmeric and its principal bioactive curcumin on human health: Pharmaceutical, medicinal, and food applications: A comprehensive review. *Frontiers in Nutrition*, *9*, 1040259. https://doi.org/10.3389/fnut.2022.1040259

Fadhilah, F. R. (2019). UJI DAYA HAMBAT PERTUMBUHAN BAKTERI Escherichia coli MENGGUNAKAN EKSTRAK RIMPANG KUNYIT Curcuma domestica val. *Jurnal Kesehatan Rajawali*, *9*(2), 35–45. https://doi.org/10.54350/jkr.v9i2.33

Hafizah, I., Akib, N. I., & Fajrianto, M. (2015). Uji Aktivitas Antibakteri Ekstrak Metanol Rumput Laut (Eucheuma sp) Pada Berbagai Tingkat Konsentrasi Terhadap Pertumbuhan Bakteri Escherichia coli Dan Staphylococcus aureus. *Jurnal Ilmiah Fakultas Kedokteran Universitas Halu Oleo*, *1*(2), 64–70. http://ojs.uho.ac.id/index.php/medula/article/view/194

Hamiyati, H., & Laratmase, A. J. (2021). Pengembangan Pengetahuan Tanaman Obat Herbal dengan Perilaku Bertanggung Jawab Mahasiswa terhadap Lingkungan Universitas Negeri Jakarta. *Jurnal Green Growth Dan Manajemen Lingkungan*, *10*(2), 59–64. https://doi.org/10.21009/jgg.102.101

Hardian Nugro Astuti, R., Samadi, K., & Priyo Prasetyo, E. (2016). Daya Antibakteri Ekstrak Daun Belimbing Wuluh (Averrhoa bilimbi linn) terhadap Bakteri Enterococcus faecalis. *Conservative Dentistry Journal*, *6*(2), 93–98.

Kali, A., Bhuvaneshwar, D., Charles, P. M. V, & Seetha, K. S. (2016). Antibacterial synergy of curcumin with antibiotics against biofilm-producing clinical bacterial isolates. *Journal of Basic and Clinical Pharmacy*, *7*(3), 93–96. https://doi.org/10.4103/0976-0105.183265

Lestari, H. D., & Asri, M. T. (2021). Aktivitas Antibakteri Ekstrak Kulit Buah Kakao (Theobroma cacao L.) Terhadap Staphylococcus epidermidis. *LenteraBio : Berkala Ilmiah Biologi*, *10*(3), 302–308. https://doi.org/10.26740/lenterabio.v10n3.p302-308

Otto, M. (2009). Staphylococcus epidermidis--the "accidental" pathogen. *Nature Reviews. Microbiology*, *7*(8), 555–567. https://doi.org/10.1038/nrmicro2182

Pangemanan, A., . F., & Budiarso, F. (2016). Uji daya hambat ekstrak rimpang kunyit (Curcuma longa) terhadap pertumbuhan bakteri Staphylococcus aureus dan Pseudomonas sp. *Jurnal E-Biomedik*, *4*(1). https://doi.org/10.35790/ebm.4.1.2016.10840

Yuliati, Y. (2017). UJI EFEKTIVITAS EKSTRAK KUNYIT SEBAGAI ANTIBAKTERI DALAM PERTUMBUHAN Bacillus sp dan Shigella dysentriae SECARA IN VITRO. *Jurnal Profesi Medika : Jurnal Kedokteran Dan Kesehatan*, *10*(1). https://doi.org/10.33533/jpm.v10i1.11