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Antibacterial Activity of Graptophyllum pictum (L.) Griff Extract using Variations of Ultrasonic Frequency against *Escherichia coli*

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Abstract

Background: *Escherichia coli* is a Gram-negative bacteria and is the main suspect for urinary tract infections and gastroenteritis. The cases of antibiotic resistance against *E. coli* continue to increase due to public non-compliance with taking antibiotics. Purple leaf (*Graptophyllum pictum (L.*) Griff) is a plant that grows widely in Indonesia and is generally used as a herbal medicine. This plant contains alkaloids, flavonoids, tannins, and steroids, which can inhibit bacterial growth. This content is obtained through an extraction process. This study aimed to determine the difference in effectiveness of purple leaves extracted using the Ultrasound-Assisted Extraction method at a frequency of 30 kHz, 40 kHz, and 50 kHz against the growth of *E. coli*. **Methods:** This research is purely experimental and was carried out in vitro. The antibacterial activity test used the suitable diffusion method, and then the diameter of the inhibition zone was measured. This research was analyzed using the Kruskal-Wallis and Mann-Whitney tests, which resulted in significant differences between treatment groups. **Results:** The average diameter of the inhibition zone produced at frequencies of 30 kHz, 40 kHz, and 50 kHz is 3,40 mm, 6,22mm, and 4,37mm. **Conclusions:** The results of this research show that the most optimal frequency is 40 kHz; this frequency can form cavitation in purple leaf cells, thereby producing phytochemical substances that can damage the cell structure of *E. coli*.

Keywords: Antibacterial; *Escherichia coli*; Purple leaves; Ultrasound Assisted Extraction

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Introduction

Escherichia coli is a Gram-negative bacterium most often found in many bacterial infectious diseases at all ages. This bacteria is a coccobacillus, does not have spores, is a facultative anaerobe, and is part of the Enterobacteriaceae family. The human intestine is a residence for *E. coli*. Still, in conditions of abnormal bacterial growth, such as lack of concern for sanitation, these bacteria can enter the digestive tract through the mouth and cause gastroenteritis, characterized by diarrhea symptoms (Murray et al., 2020). *E. coli* was found to be the main bacteria causing diarrhea in 29.53% of cases (Muziburrahman et al., 2022).

Gastroenteritis, or stomach flu, is an infectious diarrhea that can be found at all ages, but this disease is generally found in children aged 5-17. This infection is often accompanied by dehydration, causing 2 million deaths every year throughout the world, especially in developing countries, which tend to have inadequate health facilities (Plidikoua et al., 2023). Based on the 2018 Basic Health Research (Riskesdas) report, the prevalence of diarrhea in Indonesia was 8.1% in all age groups, and in toddlers, it was 12.7% (Riskesdas, 2018).

Apart from that, E.coli is also the main suspect in urinary tract infections (UTI). This bacteria is found in at least 80% of UTI cases (McAninch & Lue, 2020). Fatmawati Central General Hospital recorded an increase in UTI cases by 6.67% in 2020 (Hanung, 2020). UTI cases in Indonesia number 90-100 cases per 100,000 population yearly or around 180,000

cases yearly (Department of Health of the Republic of Indonesia, 2014). Women have a higher risk of experiencing UTIs, and around 50% of women who have been infected will experience recurrent UTIs (McAninch & Lue, 2020). UTI can be found at any age, from neonates to older adults, with a better prognosis if treated in the acute phase using adequate therapy.

The commonly used therapies for diarrhea and UTI are the antibiotics ciprofloxacin, metronidazole, cephalosporin, and trimethoprim. However, public compliance and awareness regarding the use of antibiotics is deficient, so cases of antibiotic resistance in the community continue to increase (Wulandari & Rahmawardany, 2022). Cases of antibiotic resistance require more attention to the subsequent therapy that must be chosen, such as herbal medicines. Nowadays, people still often use herbal medicines to treat diarrhea and even UTIs. The choice of types of herbal medicine is very diverse, one of which is purple leaf (*Graptophyllum pictum (L.*) Griff) (Wu et al., 2021).

Purple leaf is a plant from Papua New Guinea and is a member of the Acanthaceae. People generally use these leaves as a medicine for hemorrhoids, skin diseases, kidney stones, and infectious diseases, such as tonsillitis. This medicinal plant contains several chemical compounds, such as alkaloids, flavonoids, saponins, steroids, and tannins, which are known to have antibacterial properties (Retnaningsih et al., 2019). Apart from that, this medicinal plant is an ornamental plant because it is easy to plant and care for. This plant is also often sold and used in extract form (Amelia, 2019). Macerated extract of purple leaves inhibited the development of *E. coli* bacteria (Makkiyah et al., 2021).

Extracts are made using various methods, from conventional methods (maceration, inundation, etc.) to modern methods (Ultrasound-Assisted Extraction and Microwave Assisted Extraction). This research will use a modern extraction method, namely ultrasound-assisted (UAE) or ultrasonic extraction. This ultrasonic extraction tool uses ultrasonic energy to extract bioactive plant compounds and can form extracts quickly (Kumar et al., 2020).

Method

This research method is an experimental study with observations made in vitro. This study contained five groups of samples, namely negative control (Aquadest), positive control (Amoxisilim 500 mg), and ultrasonic extract of purple leaves at frequencies of 30 kHz, 40 kHz, and 50 kHz.

Making Extracts

Purple leaves (*Graptophyllum pictum*) obtained from the Pharmaceutical Laboratory of FK UPNVJ (determination data in Figure 1) were weighed as much as 500 grams, washed, and dried. After that, it is crushed into powder.

Fifty grams of purple leaf Simplicia, which was already in powder form, was weighed and put into an Erlenmeyer tube, then 70% ethanol solvent was poured into the tube with a ratio of Simplicia and solvent of 1:5. The Erlenmeyer tube was inserted into an ultrasonic water bath which has been adjusted based on the desired frequency at a temperature of 45^{o}C for 20 minutes, as in figure 1. Next, filtering is carried out using filter paper to produce the filtrate. The resulting filtrate was evaporated using a rotary evaporator at a temperature of 70^{o}C to make a thick extract of purple leaves with a concentration of 100%. Then, the yield weight was calculated as written in Table 1. The thick purple leaf extract was diluted using distilled water to produce an extract with a concentration of 70%.

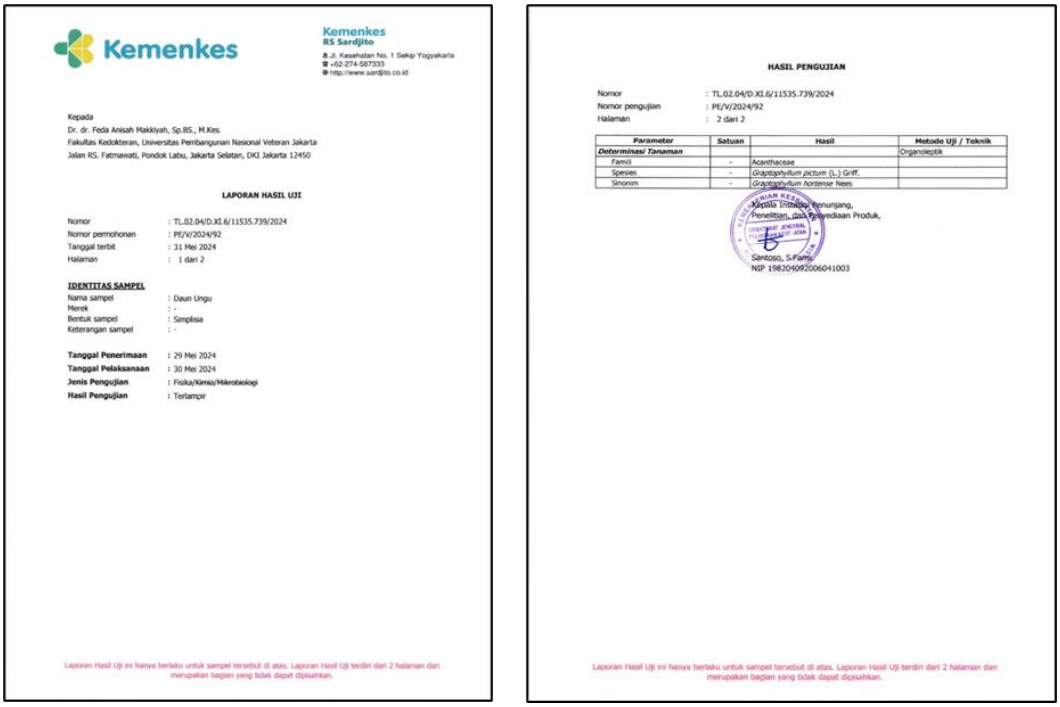

Figure 1. Purple Leaf Determination Results

The yield calculation was carried out for purple leaf extract to determine the amount of extract obtained. This calculation can be seen in Table 1 below.

Table 1. Extract Yield Results

Temperature	Time	Frequency (kHz)	Extract Weight (gram)	Yield Weight (%)
45°C	20 minutes	30	21,1	42,2
		40	23,9	47,8
		50	17,0	34,0

Figure 1. Ultrasonic extraction process

Antibacterial Activity Test

The E.coli bacterial suspension will be standardized using the 0.5 McFarland standard. One dose of the bacterial isolate from McConkey Agar media is taken. Then, it dissolves in 2 mL of NaCl solution until the turbidity is the same as the Mac Farland standard. The antibacterial test was carried out using the excellent diffusion method of Nutrient Agar (NA) media in petri dishes. The thriving press is made in two layers, namely, the first layer is pure NA, and the second layer is NA mixed with *E. coli* suspension; then, a well is formed using a cylindrical plate. Each well will be dripped with the control group and purple leaf ultrasonic extract at 30 kHz, 40 kHz, and 50 kHz with an extract concentration of 70%, 20 µL each. After

dripping, the cup was incubated for 24 hours at 37°C. The inhibitory ability of the extract was measured using a caliper on the clear zone found around the well. The ultrasonic extract of purple leaves was found to produce an inhibition zone, and the antibacterial test results showed differences in each sample group, as recorded in Table 3.

Data Analysis

This study has five groups of data (> 2 groups of data), which are independent, normally distributed, and homogeneous data groups so that a comparative test (One-Way ANOVA) can be used, namely to test the average and difference in the diameter of the inhibition zone produced at the frequency different ultrasonics. If there is a significant difference in the mean zone of inhibition ($p<0.05$), then the Post Hoc test is continued to determine the most crucial treatment group. The requirements for the One-Way ANOVA test in this study were unmet, so an alternative test was used, namely the Kruska-Wallis Test, and continued with the Mann-Whitney test. The results of the Kruskal-Wallis test are listed in Table 2 (Pramono et al., 2022).

Table 2. Kruskal-Wallis Test

	Inhibition Zone Diameter
Kruskal-Wallis	22,422
Asymp. Sig.	0,000

Result

Table 3. Inhibition Zone Measurement Results

Experiment	**Ultrasonic Frequency**			**Positive Control**	**Negative Control**
	30 kHz	**40 kHz**	**50 kHz**		
1	4,25	5,74	4,54	42,63	0,00
2	2,59	6,24	3,28	54,38	0,00
3	2,52	7,33	4,47	46,90	0,00
4	4,11	6,41	4,23	41,62	0,00
5	3,55	5,41	5,34	37,17	0,00
Average	**3,40**	**6,22**	**4,37**	**44,54**	**0,00**

The ultrasonic extract of purple leaves at frequencies of 30 kHz, 40 kHz, and 50 kHz, as well as the positive control and negative control, had their inhibition zones measured and the average measured, recorded in Table 2 and documentation of the results after incubation after 24 hours is in Figure 2.

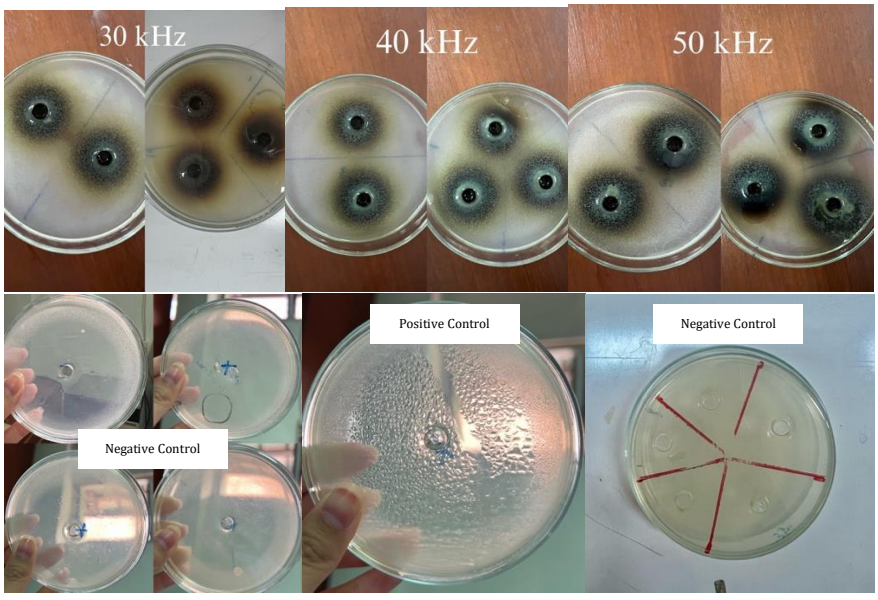

Figure 2. Inhibition Zone Results After 24 Hours of Incubation.

Discussion

The results of this research show that the average diameter of the inhibition zone produced by ultrasonic extract of purple leaves at frequencies of 30 kHz, 40 kHz, and 50 kHz in *Escherichia coli* cultures using Nutrient Agar is 3.40 mm, respectively; 6.22mm; 4.37mm. Based on previous research, making extracts using the Ultrasound-Assisted Extraction (UAE) method can produce extracts with the ability to inhibit the activity of *E. coli* bacteria (Ardianti & Kusnadi, 2014; Maligan et al., 2016; Safarpoor et al., 2018; Anggraini et al., 2019; Tungmunnithum et al., 2019).

The diameter of the inhibitory zone is categorized by Davis and Stout into four groups, namely an inhibitory zone diameter ≥ 20 mm, meaning the inhibitory power is categorized as very strong, an inhibitory zone diameter of 10 - 20 mm is classified as an inhibitory solid power, an inhibitory zone diameter of 5-10 mm is categorized as a medium inhibitory power, and a diameter ≤ 5 mm is classified as weak inhibition (Sangkoy et al., 2023). This study's 30 kHz and 50 kHz frequencies produced weak inhibitory power, while the 40 kHz frequency produced moderate inhibitory power.

The normality test in the treatment group obtained a P value >0.05, which indicates the group data was normally distributed. The homogeneity test obtained a P value <0.05, meaning the data variance was not homogeneous. Therefore, the One-Way ANOVA test could not be used because it did not meet the requirements, so the researchers used the alternative Kruskal-Wallis test to compare the inhibition zones between treatment groups. The Kruskal-Wallis test in this study showed that at least one group differed significantly from other treatment groups. Next, the Mann-Whitney test was carried out, resulting in significant differences between treatment groups with a P value <0.05 in the 30 kHz, 40 kHz, 50 kHz, positive and negative control groups. However, in the 30 kHz and 50 kHz groups, there was no significant difference in the diameter of the inhibition zone, where both treatment groups had inhibition power that was categorized as weak.

The ability to inhibit the antibacterial activity of purple leaf (*Graptophyllum pictum (L.) Griff*) ultrasonic extract can be seen through the inhibition zone formed around the well. This study found inhibition zones around the wells with varying diameters in each treatment group. The inhibition zone in *E. coli* can be produced due to the active phytochemical compounds in purple leaves. The phytochemical content based on the phytochemical test of purple leaf extract consists of alkaloids, flavonoids, steroids, tannins, and phenols. Alkaloids and tannins work as antibacterial substances by inhibiting protein synthesis and damaging bacterial cell walls; flavonoids and steroids can change bacterial protein components and increase bacterial cell permeability, leading to bacterial cell damage. Phenol damages cell membranes, causing bacterial metabolites to leak (Amelia, 2019).

In this study, purple leaf extract was made using the ultrasound-assisted extraction (UAE) method, which, in previous research, was effective in producing extracts faster using wave frequencies above 20 kHz (Kumar et al., 2020). The 30 kHz frequency in this study created an inhibitory zone for the growth of *E. coli*; this is in line with research by Tungmunnithum et al. (2019), where the study stated that miana leaf extract at a frequency of 30 kHz could produce active compounds that act as antibacterials. Frequencies below 40 kHz can have a cavitation effect on the structure of orange peel cells, although the cavitation intensity is not as strong as the 40 kHz frequency (Chen et al., 2021).

Research by Ardianti & Kusnadi (2014) & Safarpoor et al. (2018) stated that ultrasonic extracts of *Crescentia cujete Linn* and *Sylibum marianum* at a frequency of 40 kHz were able to produce an inhibition zone against *E. coli* with moderate and inhibitory solid power. This is in line with this research, namely that the largest average diameter of the inhibition zone was found at a frequency of 40 kHz. This frequency shows the most potent ability to form cavitation in the orange peel cell structure, influencing the amount of final extract produced and the amount of phytochemical content diffused into the solvent (Chen et al., 2021).

The 50 kHz frequency can inhibit the activity of *E. coli* bacteria based on the inhibition zone produced, and this is in line with research by Maligan et al. (2016), which states that ultrasonic microalgae extract at a frequency of 50 kHz can inhibit the growth of *E. coli* based

on the resulting zone of inhibition. This frequency is the most prominent frequency used in this study. Still, the diameter of the resulting inhibition zone does not show an increase that exceeds the average diameter at a frequency of 40 kHz. This was explained in research by Dzah et al. (2020), which stated that increasing the ultrasonic frequency did not affect the number of chemical compounds produced in berry extracts.

The UAE method is influenced by several factors, namely frequency, extraction temperature, and extraction time (Dzah et al., 2020). The power of ultrasonic waves affects the efficiency of the extraction process by speeding up the diffusion process of target compounds into the solvent and helping the process of destroying plant cells; however, high ultrasonic frequencies can cause excessive degradation of plant compounds, which can reduce extraction results (Tungmunnithum et al., 2019). The use of temperature affects reducing viscosity and pressure on the plant surface, which destroys the simplicial cell structure, and the length of extraction time determines the size of exposure to ultrasonic waves on the target substance (Sekarsari et al., 2019; Ummat et al., 2020; Kristina et al., 2022). The temperature chosen for making the extract for this research was 45^{o}C, which was the most optimal for producing active compounds that can inhibit bacterial synthesis (Sekarsari et al., 2019).

Conclusions

Purple leaf extract extracted using the Ultrasound-Assisted Extraction (UAE) method can inhibit the activity of *Escherichia coli* bacteria in vitro by producing different average diameters of inhibition zones. The average inhibition zone produced at a frequency of 30 kHz is 3.40 mm; at a frequency of 40 kHz, it is 6.22 mm; at a frequency of 50 kHz, it is 4.37 mm so that the largest average diameter of the inhibition zone is obtained at a frequency of 40 kHz.

Declaration statement

The authors reported no potential conflict of interest.

References

Amelia, O. (2019). Pemanfaatan Daun Ungu ((Graptophyllum pictum) Sebagai Bahan Dasar Pewarna Alami. *Jurnal Teknologi Agroindustri*, *11*(2), 34. https://doi.org/10.46559/tegi.v11i2.5780

Anggraini, W., Choirun Nisa, S., Ramadhani, R. DA, & Ma'arif, B. (2019). Aktivitas Antibakteri Ekstrak Etanol 96% Buah Blewah (Cucumis melo L. var. cantalupensis) terhadap pertumbuhan bakteri *Escherichia coli*. *Pharmaceutical Journal of Indonesia 2019*, *5*(1), 61–66.

Ardianti, A., & Kusnadi, J. (2014). Ekstraksi Antibakteri dari Daun Berenuk (Crescentia cujete Linn.) Menggunakan Metode Ultrasonik. *Jurnal Pangan Dan Agroindustri*, *2*(2), 28–35.

Chen, T. T., Zhang, Z. H., Wang, Z. W., Chen, Z. L., Ma, H., & Yan, J. K. (2021). Effects of ultrasound modification at different frequency modes on physicochemical, structural, functional, and biological properties of citrus pectin. *Food Hydrocolloids*, *113*. https://doi.org/10.1016/j.foodhyd.2020.106484

Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound-assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. In *Food Bioscience* (Vol. 35). Elsevier Ltd. https://doi.org/10.1016/j.fbio.2020.100547

Hanung, M. S. (2020). *Rencana Strategi Bisnis Taun 2020-2024 RSUP Fatmawati*.

Kristina, C. V. M., Yusasrini, N. L. A., & Yusa, N. M. (2022). Pengaruh Waktu Ekstraksi Dengan Menggunakan Metode Ultrasonic Assisted Extraction (UAE) Terhadap Aktivitas Antioksidan Ekstrak Daun Duwet (Syzygium cumini). *Itepa: Jurnal Ilmu Dan Teknologi Pangan*, *11*(1), 13–21.

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2020). Ultrasound-assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrasonics Sonochemistry*, *70*(105325). https://doi.org/10.1016/j.ultsonch.2020.105325

Makkiyah, F., Rahmi, E. P., Revina, R., Susantiningsih, T., & Setyaningsih, Y. (2021). Graptophyllum pictum (l.) griff. (syn: *Justicia picta linn*.) and its effectiveness: A well-known Indonesian plant. *Pharmacognosy Journal*, *13*(3), 835–838. https://doi.org/10.5530/pj.2021.13.106

Maligan, J. M., Adhianata, H., & Zubaidah, E. (2016). Production and Identification of Antimicrobial Compounds from Microalgae Tetraselmis chuii with UAE Method (Study Type of Solvent and Total Cycle Extraction). *Jurnal Teknologi Pertanian*, *17*(3).

McAninch, J. W., & Lue, T. F. (2020). *Smith & Tanagho's General Urology* (Vol. 19).

Murray, P. R., Rosenthal, K. s, & Pfaller, M. A. (2020). *Medical Microbiology Murray 8th Ed* (8th ed.). Elsevier.

Muziburrahman, M.-, Husada, D., & Utomo, B. (2022). Identification of Bacteria Causing Diarrhea in Under-fives Children Using Culture Methods in Bima, Indonesia. *Jurnal Berkala Epidemiologi*, *10*(1), 95. https://doi.org/10.20473/jbe.v10i12022.95-102

Pramono, A. P., Amru, B. A., Rahmani, H. A., Fernanda, S. A., Nugraha, Y., Ramadhan, M. Y. A., Harahap, A. F. P., Fauzantoro, A., Irsyad, N. S., Bahar, M., Puspita, O. S., Zulfa, F., Yati, K., Jufri, M., & Gozan, M. (2022). Nicotiana tabacum var. Virginia Bio Oil-based Pyrolisis Extraction Have Prominence Antimicrobial Potential Compared to Ethanol Heat Reflux Extraction (EHRE). *HAYATI Journal of Biosciences*, *29*(4), 515–525. https://doi.org/10.4308/hjb.29.4.515-525

Plidikoua, A., Ziem, O., Ngom, J. T., Mamboune, A., Clémence, B. A., Koro, F. K., & Ngane, R. A. N. (2023). Diarrheic *Escherichia coli*: A Predominant Etiological Agent of Gastroenteritis, a Case Study in Douala, Cameroon. *Journal of Biosciences and Medicines*, *11*(06), 1–17. https://doi.org/10.4236/jbm.2023.116001

Retnaningsih, A., Primadiamanti, A., & Febrianti, A. (2019). Uji Daya Hambat Ekstrak Etanol Daun Ungu (Graptophyllum pictum (L.) griff) terhadap Bakteri Staphylococcus epidermidis dan bakteri Propionibacterium acnes Penyebab Jerawat dengan Metode Cakram. *Jurnal Analisis Farmasi*, *4*(1), 1–9.

Riskesdas. (2018). *Laporan Nasional Riskesdas 2018 Kementerian Kesehatan RI*.

Safarpoor, M., Ghaedi, M., Asfaram, A., Yousefi-Nejad, M., Javadian, H., Zare Khafri, H., & Bagherinasab, M. (2018). Ultrasound-assisted extraction of antimicrobial compounds from Thymus daenensis and Silybum marianum: Antimicrobial activity with and without the presence of natural silver nanoparticles. *Ultrasonics Sonochemistry*, *42*, 76–83. https://doi.org/10.1016/j.ultsonch.2017.11.001

Sangkoy, W. J., Simbala, H. E. I., & Rumondor, E. M. (2023). Aktivitas Antibakteri Ekstrak Etanol Daun Pinang Yaki (Areca vestiaria) Terhadap Bakteri Staphylococcus aureus, *Escherichia coli*, dan Pseudomonas aeruginosa. *Pharmacon*, *12*(1), 133–139.

Sekarsari, S., Rai Widarta, W., Agung, A., Ngurah, G., & Jambe, A. (2019). Pengaruh Suhu dan Waktu Ekstraksi Dengan Gelombang Ultrasonik terhadap Aktivitas Antioksidan Ekstrak Daun Jambu Biji P. *Jurnal Ilmu Dan Teknologi Pangan*, *8*(3), 267–277.

Tungmunnithum, D., Garros, L., Drouet, S., Renouard, S., Lainé, E., & Hano, C. (2019). Green ultrasound-assisted extraction of trans rosmarinic acid from plectranthus scutellarioides (L.) R.Br. leaves. *Plants*, *8*(3). https://doi.org/10.3390/plants8030050

Ummat, V., Tiwari, B. K., Jaiswal, A. K., Condon, K., Garcia-Vaquero, M., O'Doherty, J., O'Donnell, C., & Rajauria, G. (2020). Optimisation of ultrasound frequency, extraction time and solvent for the recovery of polyphenols, phlorotannins and associated antioxidant activity from brown seaweeds. *Marine Drugs*, *18*(5). https://doi.org/10.3390/md18050250

Wu, D., Ding, Y., Yao, K., Gao, W., & Wang, Y. (2021). Antimicrobial Resistance Analysis of Clinical *Escherichia coli* Isolates in Neonatal Ward. *Frontiers in Pediatrics*, *9*(670470). https://doi.org/10.3389/fped.2021.670470

Wulandari, A., & Rahmawardany, C. Y. (2022). Perilaku Penggunaan Antibiotik di Masyarakat. *Sainstech Farma Jurnal Ilmu Kefarmasian*, *15*(1).