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Article

Testing the Antibacterial Activity of Red Ginger Essential Oil and Red Ginger Methanol Extract

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Abstract. The red ginger plant is a spice plant used in traditional medicine and has the potential to be antibacterial. This research aims to isolate and identify red ginger essential oil, extract red ginger, and test antibacterial activity against Staphylococcus aureus and Escherichia coli bacteria. Red ginger essential oil is obtained by steam distillation, while red ginger extract is obtained using the maceration method with methanol solvent. Red ginger essential oil yielded 0.13%, and red ginger extract was 12%. The phytochemical test results of red ginger essential oil contain tannin and sopinin, while red ginger extract contains flavonoids, tannin and saponin. The results of analysis using GCMS show that there are 31 components of red ginger essential oil, with the main compounds 2-butanone-4-(3-hydroxy-2-methoxyphenyl) (21.51%) and Zingerone (15.10%). The GCMS test results for red ginger methanol extract obtained 31 components, with the main components being citral (24.05%). The results of antibacterial testing of red ginger extract did not provide activity in inhibiting bacteria, whereas red ginger essential oil at a concentration of 100% provided inhibition of the growth of E. coli bacteria (14.00 ± 2.00 mm) and at a concentration of 40% for S. aureus bacteria (22.67 ± 2.31 mm).

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1. Introduction

Along with the emergence of various kinds of diseases today, various research has been encouraged to find solutions that can be used to overcome these problems. Diseases are generally divided into diseases caused by infection with microorganisms and degenerative diseases. Diseases caused by microorganism infections often occur due to human interaction, and one of the microorganism infections is bacteria. Bacteria that can cause disease include Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) [1-2].

S. aureus bacteria are bacteria that can become pathogenic (infectious) if the number is abnormal [3-4], and pus will appear due to damage to body tissue, which is one of the symptoms of infection [5-6]. S. aureus bacteria can enter the human body through hair follicles, sweat glands, skin, nostrils or small wounds [7-8]. Diseases caused by S. aureus bacteria include boils, acne, and impetigo, and parts of the body where S. aureus is known to be present are the skin, nasopharynx, and intestines [9]. Apart from S. aureus bacteria, there are also E. coli bacteria, which are dangerous for the host organism. Comparative analysis shows that avian and human E. coli isolates contain similar gene sets; both are zoonotic and belong to the same phylogenetic group [10]. This bacteria can be dangerous because of its presence in the human body, especially in the digestive tract and intestines [11-12]. Diseases caused by E. coli bacteria include diarrhea, vomiting, nausea, urinary tract infections, and pyogenic infections [13-14]. However, some E. coli strains can cause intestinal and extraintestinal infections, such as urinary tract infections, respiratory tract infections, meningitis, and sepsis [15].

Treatment that can be done to treat diseases caused by bacterial infections is by taking antibiotics and traditional treatment. The use of antibiotics is a method that is often used. However, if this treatment is given excessively over a long period and without a doctor's recommendation, it can be dangerous because bacterial resistance will occur [16]. Treatment and prevention of disease using traditional methods, namely with natural ingredients, one of which is ginger plants [17] and ginger peel [18]. The ginger plant is a spice plant, the same as several other spice plants such as turmeric, ginger, galangal and galangal, which are traditional medicines [19-20]. Ginger provides health benefits to humans due to its biological activities such as antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antihyperlipidemic, antiobesity, and hepatoprotective activities [21].

Ginger is effective in reducing NVP disease, digestive function, increasing the expression level of colorectal cancer risk markers, and anti-inflammatory function [22]. Ginger is known to have bioactive compounds, such as phenolic compounds, flavonoid compounds, and essential oils, which provide pharmacological activity [23]. Gingerol is the main phenolic compound in a ginger rhizome, which consists of gingerol, shogaol, paradol, zingerol, gingerones, and gingerdiones. The red ginger plant (Zingiber officinale var rubrum) is used as a traditional medicine because its essential oil and oleoresin content is higher than the other two types [24]. Red ginger contains vanilloid compounds, monoterpenes, sesquiterpenes, diterpenes, flavonoids, amino acids, etc [25].

Red ginger extract was able to inhibit the growth of K. pneumoniae at a concentration of 125 µg/mL each with a decrease in absorption values before and after incubation. The higher the concentration of the red ginger ethanol extract mixture, the higher the leakage of K. pneumoniae bacterial cells [26]. Ginger essential oil showed significant antibacterial activity against S. putrefaciens, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 2.0 and 4.0 µL/mL, respectively [27]. Geographical location, plant collection time and chemical components in red ginger influence its pharmacological effectiveness [28]. The essential oil contained in red ginger from Maluku is known to have antibacterial benefits, but the antibacterial activity of the extract from red ginger is not yet known. This research aims to isolate and identify red ginger essential oil, extract red ginger and test antibacterial activity against S.aureus and E.coli bacteria.

2. Experimental Section

2.1. Isolation of Red Ginger Essential Oil

6 kg of dried red ginger rhizomes were weighed and placed on a filter in a distillation kettle filled with water. Distillation is carried out for 7 hours until the essential oil is obtained; the distillate obtained is then collected in a separating funnel. The oil obtained is then separated from the water mixture, and anhydrous Na_2SO_4 is added. The percentage of the yield of the distilled essential oil is calculated and stored for further analysis.

2.2. Red Ginger Extraction

Weigh 0.5 kg of dried red ginger rhizome, put it in an extraction bottle and add 95% methanol. The maceration process is carried out for 5×24 hours, after which it is filtered, and a filtrate is obtained, which will be evaporated again with the solvent. The percentage of red ginger extract resulting from maceration was calculated and stored for further analysis.

2.3. Phytochemical

2.3.1 Test Phenolic Test

A total of 2 mL of each oil sample and red ginger extract was put into a test tube, then ten drops of 1% $FeCl_3$ were added and then shaken. The test is positive if solid blue, green, red, purple and black are produced.

2.3.2 Flavonoid Test

A total of 2 mL of each oil sample and red ginger extract was put into a test tube, and then 0.5 g of Mg powder and 1 mL of 1% HCl were added. The test is positive if it produces foam and a red, orange or red colour. If an orange-yellow colour occurs, it indicates the presence of flavones, chalcone, and aurone.

2.3.3 Tannin Test

A total of 2 mL of each oil sample and red ginger extract was put into a test tube, then 1 mL of distilled water was added and boiled. The mixture was filtered, and the filtrate was added with three drops of 1% $FeCl_3$ and shaken. The test is positive if it produces a dark green-brown or dark blue.

2.3.4 Steroid Test

A total of 2 mL of each oil sample and red ginger extract was put into a test tube, and then ten drops of glacial acetic acid and ten drops of concentrated sulfuric acid were added. The test is positive if it produces a blue or green colour. Each colour produced has a different concentration found in the sample.

2.3.5 Saponin Test

2 mL each of the oil and red ginger extract samples were put into a test tube, and then three drops of 2 N HCl were added. The test was positive if it produced foam.

2.4. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The results of the red ginger oil in the distillation and extract process were analyzed for content using an Agilent 7890 – 5975 Gas Chromatography-Mass Spectroscopy tool.

2.5. Antibacterial Testing Uses the Well Method

The antibacterial test using the well method was carried out in 2 stages, namely the stage to test the antibacterial activity of red ginger essential oil and red ginger extract. The antibacterial activity test uses Escherichia coli and Staphylococcus aureus bacteria, purified and rejuvenated first in the

laboratory. The next stage was to make five concentration variations (20, 40, 60, 80, and 100%) of red ginger essential oil and red ginger extract. A two-layer agar nutrient medium was used in this testing process. The first layer of the medium is a nutritious base layer.

In contrast, the second layer is a mixture of media and pathogenic bacterial suspension with a cell density of 10^6 CFU/ml. The top of the media (second layer) was drilled with holes with a diameter of 6–7 mm using a sterile punch. Each petri dish was made with five wells. Next, 40 µl of the oil and red ginger extract suspension was carried out and dropped into three wells. In comparison, 40 µl of distilled water was given to 1 well as a negative control and 40 µl of liquid amoxicillin antibiotic was given to 1 well as a positive control. The agar medium was incubated at 37 °C for 24 hours. Measure the diameter of the inhibition zone formed after inoculation.

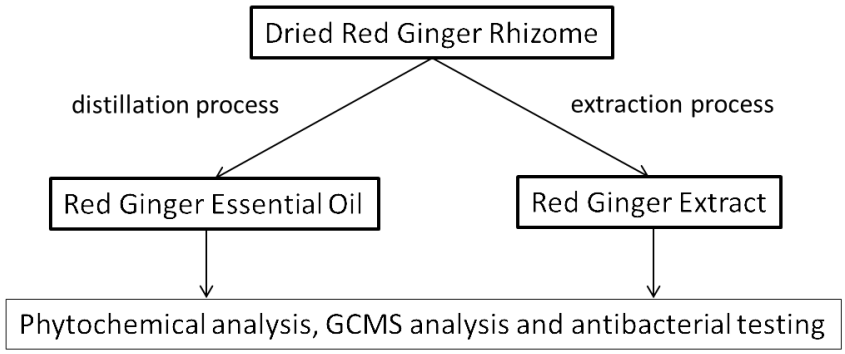

Figure 1. Research method flow chart

3. Results and Discussion

The red ginger essential oil obtained a yield of 0.13%, and the red ginger extraction obtained a yield of 12%. Good quality red ginger essential oil with a high level of purity obtained a soak of 2.1 - 2.6% [29]. Phytochemical testing carried out on the essential oil and methanol extract of red ginger was found to contain secondary metabolite compounds. The results of the phytochemical testing are shown in Table 1. The results of the qualitative test on red ginger oil showed that it contained tannin and saponin compounds, while the results of the qualitative test on red ginger extract showed The content of flavonoids, tannins and saponins was obtained. The results of this research are in line with research conducted by Wahid [30] which showed that red ginger rhizomes contain flavonoid, phenolic and terpenoid compounds.

Table 1. Results of phytochemical testing of essential oils and methanol extract of red ginger

No	Test type	Red ginger essential oil	Red ginger extract	Description of red ginger essential oil	Description of red ginger methanol extract
1	Phenolic	-	-	Light brown colour	Brown
2	Flavonoids	-	+	White color	Orange, red and foamy
3	Tannin	+	+	Dark brown colour	Dark brown colour
4	Steroids	-	-	Mastic	Orange, red colour
5	Saponins	+	+	Clear and foamy colour	The brownish-red color and foamy

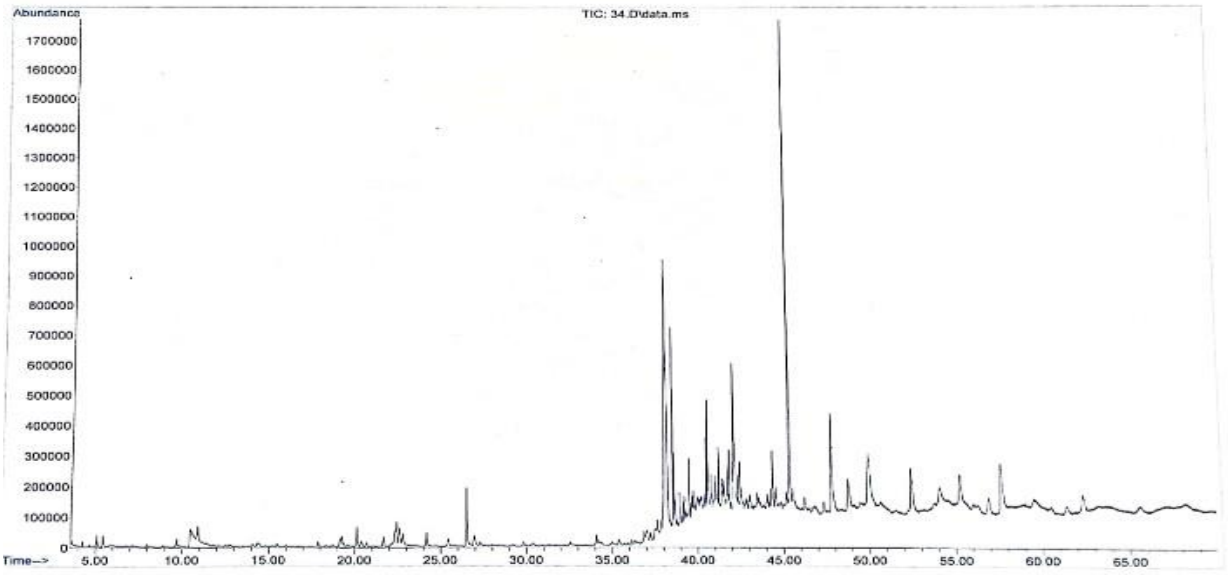

Figure 2. Results of GC-MS analysis of red ginger essential oil

The results of GC-MS analysis of red ginger essential oil obtained 31 peaks (Figure 2), which shows the presence of 31 components. The main components of red ginger essential oil are nine compounds, which are presented in Table 2. The main component of red ginger essential oil from Maluku is Zingerone (21.51%), 2-Butanone-4-(4-hydroxy-3-methoxyphenyl) (15.10%), Palmitic acid (8.36%), oleic acid (5.86%), and n-Hexadecanoic acid (5, 64%). Research conducted by Tritanti [29], shows that the main components of red ginger essential oil include E-Cital, Chempene, Cineole and Zingiberene. Badrunanto [31] found that the main component of red ginger essential oil was geranial (28.3%). The main components of ginger essential oil from Thailand are camphene, 3-carene, o-cymene, caryophyllene, α-curcumene, sabinol trans, citral, and santalol. Ginger essential oils from China are α-pinene, camphene, limonene, longicyclene, copaene, longifolene, β-sesquiphellandrene, alloaromadendrene, γ-muurolene, α-curcumin, α-farnesene, and citral [32].

Table 2. Chemical components of red ginger oil

No	Retention time (Minute)	Compound	Content (%)
1	26.521	Geraniol	2.59
2	38.170	Zingerone	21.51
3	38.571	3-alil-6-metoksifenol	3.58
4	42.142	Palmitic acid	8.36
5	45.392	2-butanon-4-(4-hidroksi-3-metoksifenil)	15.10
6	47.835	n-asam heksadekanoat	5.64
7	50.004	Oleic acid	5.86
8	52.464	9(E), 11(E)-linoleic acid	4.32
9	57.705	9,2-asam oktadekadienoik (Z,Z)	4.50

Based on several previous studies that have been carried out, there are differences in the compound components contained in the red ginger tested, and this is because the red ginger samples used in this study were obtained in different places. Differences in sampling locations influence the secondary metabolite components obtained, which can be seen from soil conditions, the presence of pests, planting time, maintenance methods, and harvesting methods for red ginger [28]. The chemical composition of ginger oil is influenced by the source of the rhizome, freshness or dryness and

extraction method. Meanwhile, oleoresin which consists of gingerol, zingiberene, shogaol is classified as a non-volatile component which gives a bitter and spicy taste [33].

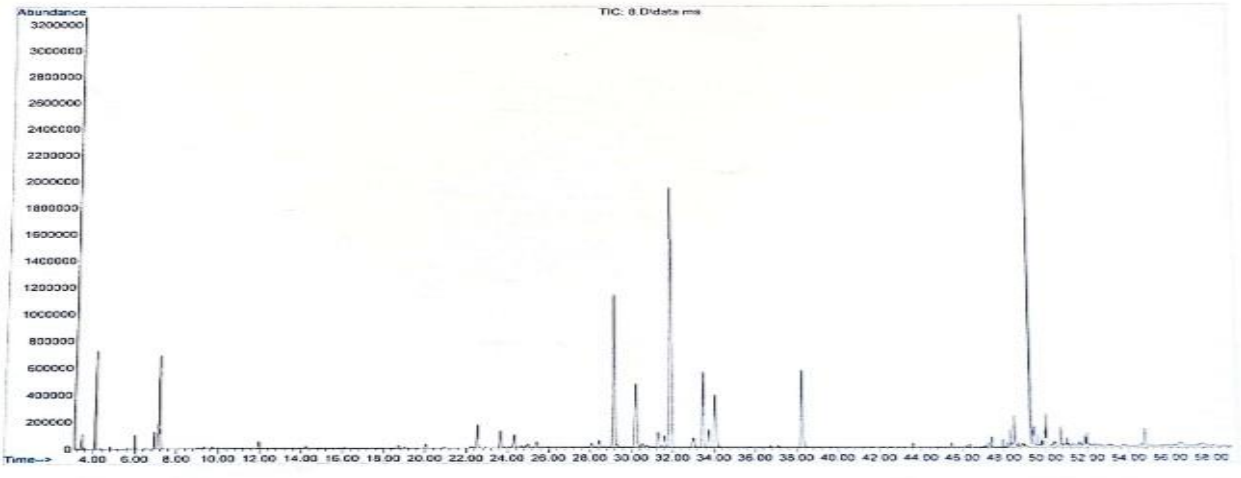

Figure 3. Results of GC-MS analysis of red ginger extract

The results of GC-MS analysis of red ginger extract obtained 31 peaks (Figure 3), which indicated the presence of 31 components. The results of this test obtained nine dominant compound components, which are presented in Table 3. The chemical components of the methanol extract of red ginger obtained 31 components, with the main components being Citral (24.05%), Chavibetol (12.82%), Neral (12.59%), Geraniol (6.48%), and Butanoic acid (5.74%). The use of liquid extraction method with alcohol solvent produces an abundant fraction with 6-gingerol. The concentration of 6-gingerol in the ethanol extract of red ginger rhizomes was 145.96 ± 1.65 ppm/µg [34].

Table 3. Chemical components of red ginger extract

No	Retention Time (Minute)	Compound	Content (%)
1	4.083	Kamfen	2.94
2	7.155	Eukaliptol	4.94
3	29.181	Neral	12.59
4	30.244	Endo-boerneol	6.72
5	31.946	Citral	24.05
6	33.477	Butanoic acid	5.74
7	34.058	Benzene	4.89
8	38.271	Geraniol	6.48
9	49.292	Chavibetol	12.82

The ingredients contained in red ginger are volatile compounds and non-volatile compounds. The classification of volatile compounds in red ginger is divided into several terpenoid compounds and essential oils. The oil obtained from this plant plays a role in providing its distinctive aroma. Non-volatile compounds consist of flavonoids and polyphenol compounds (6-gingerol and its derivatives), which function as a preventative against the presence of free radicals in the body due to their high antioxidant activity. The oleoresin, which is included in the oil, does not evaporate and is a component in giving red ginger a spicy and bitter taste [24].

Antibacterial activity testing was carried out using the well method, and the media used was NA media with Escherichia coli ATCC 8739 and Staphylococcus aureus ATCC 6538 as the test bacteria. Tests were carried out on red ginger essential oil and red ginger methanol extract with varying

concentrations (20%, 40%, 60%, 80%, 100%). Test results show that red ginger essential oil with concentrations (20%, 40%, 60%, 80%, 100%) can inhibit antibacterial activity. The inhibition of bacterial growth by red ginger oil can be seen from the bacterial inhibition zone that forms around the well. This process of inhibiting bacteria is caused because red ginger essential oil contains bioactive compounds that can interfere with or damage bacterial growth. The results of testing the antibacterial activity of red ginger essential oil and red ginger extract against S. aureus and E. coli bacteria can be seen in Table 4.

Table 4. Antibacterial activity test of red ginger essential oil and red ginger extract against S. aureus and E. coli bacteria

Bacteria	Sample concentration	Inhibition zone diameter (mm)			
		Red ginger essential oil	Red ginger extract	Positive control	Negative control
Escherichia coli ATCC 8739	20	9.33 ± 0.58	NA	26.67 ± 0.58	NA
	40	12.33 ± 0.58	NA		
	60	12.67 ± 0.58	NA		
	80	14.00 ± 0.00	NA		
	100	14.00 ± 2.00	NA		
Staphylococcus aureus ATCC 6538	20	15.67 ± 2.89	NA	42.33 ± 2.52	NA
	40	22.67 ± 2.31	NA		
	60	16.67 ± 0.58	NA		
	80	10.67 ± 1.53	NA		
	100	17.33 ± 3.06	NA		

Table 4 shows the results that the higher the concentration of red ginger essential oil given, the greater the inhibition zone power against E. coli bacteria. This can be seen from the value of the inhibitory zone getting bigger. The results of the antibacterial test were also carried out on S. aureus bacteria, where the test results also showed an inhibitory zone around the well for the test bacteria. The results of the inhibition zone of red ginger essential oil against S. aureus bacteria gave regular results, and this was indicated by the test result value of a concentration of 40%, giving data on the strength of the inhibition zone, which was greater compared to other concentrations. This process is possible due to the bacteriostatic properties of the bioactive substances contained so that because of their properties, they can only maintain bacterial growth and not stop growth or kill bacteria.

The results of observations of the inhibitory zone power of antibacterial activity for both pathogenic bacteria showed that they were able to inhibit the growth of these bacteria. Based on the results of the average activity of red ginger essential oil at a concentration of 100% with E. coli test bacteria, it gives an inhibitory zone diameter of 14.00 ± 2.00 mm and is categorized as strong in inhibiting bacterial growth, while S. aureus bacteria the zone diameter The barrier formed at a concentration of 40% was 22.67 ± 2.31 mm and could be categorized as very strong in inhibiting bacterial growth.

The antibacterial activity was categorized as very strong in inhibiting pathogenic bacteria, which was tested by administering a positive control in the form of the liquid antibiotic amoxicillin. The respective inhibitory zone power results were E. coli (26.67 ± 0.58 mm) and S. aureus (42.33 ± 2.52 mm). The compound components contained in red ginger consist of citral compounds and geraniol compounds, where these compounds act as antibacterials. Other compounds contained in ginger, such as phenols, also have the ability to denature proteins and damage cell tissue.

The results of this study show that red ginger essential oil inhibits the growth of gram-positive bacteria more than gram-negative bacteria. This is because gram-negative bacteria have the ability to maintain cell walls better than gram-positive bacteria. S. aureus has a simple cell wall, making it easy

for antibacterial compounds to enter, and the bacterium is an opportunistic pathogen [35]. Meanwhile, E. coli has a more complex cell wall, which is composed of 3 layers, namely the outer layer as a lipoprotein, the middle layer as lipopolysaccharide, which is the layer responsible for selecting incoming foreign substances and the inner layer as peptidoglycan [14].

The ability of red ginger essential oil to inhibit bacterial growth is because red ginger essential oil contains secondary metabolite compounds whose purpose can be used for antibacterial activity [36]. The compound components contained in red ginger rhizomes that have the ability to inhibit bacterial growth are phenols, flavonoids, terpenoids and essential oils. The results of the phytochemical test in this study showed that red ginger oil contains tannin and saponin compounds. Tannin's ability as an antibacterial is by precipitating proteins so that they can damage cell membranes and inhibit bacterial growth. Meanwhile, saponin compounds also play a role in inhibiting and even killing bacterial cells by damaging the permeability of cell walls.

Saponin compounds can have antibacterial properties by damaging cells, damaging cell membranes and causing important substances to leave the cell. It can also prevent the entry of important materials into the cell. If the function of the cell membrane is damaged, it will cause cell death. Testing the antibacterial activity of red ginger extract in this study showed that red ginger extract had no activity in inhibiting bacterial growth against the two test bacteria, namely S. aureus bacteria and E. coli bacteria, which were characterized by the absence of an inhibition zone.

4. Conclusion

The isolation results for red ginger essential oil were 0.13%, and the phytochemical test results contained tannin and saponin compounds. The chemical component content of red ginger essential oil contains 31 components with the main components being 2-butanone-4-(3-hydroxy-2-methoxyphenyl) (21.51%), 2-Butanone-4-(4-hydroxy-3-methoxyphenyl) (15.10%), n-hexadecanoic acid (8.36%), oleic acid (5.86%), and n-hexadecanoic acid (5.64%). The methanol extract of red ginger was 12%, and the phytochemical test results contained flavonoid, tannin and saponin compounds. The chemical components of the methanol extract of red ginger obtained 31 components, with the main components being citral (24.05%), 3-Alyl-6-methoxyphenol (12.82%), neral (12.59%), geraniol (6.48%), and Butanoic acid (5.74%). The red ginger essential oil can inhibit bacterial growth at a concentration of 100% for E. coli bacteria (14.00 ± 2.00 mm) and at a concentration of 40% for S. aureus bacteria (22.67 ± 2.31 mm).

References

[1] Breijyeh, Z., & Karaman, R. (2023). Design and Synthesis of Novel Antimicrobial Agents. *Antibiotics,* (12), 628.

[2] Zimmermann, S., Klinger-Strobel, M., Bohnert, J. A., Wendler, S., Rödel, J., Pletz, M. W., Löfer, B., & Tuchscherr, L. (2019). Clinically Approved Drugs Inhibit the Staphylococcus aureus Multidrug NorA Efux Pump and Reduce Biolm Formation. *Front*. *Microbiol,* (10), 2762.

[3] Gitirana de Santana, J. D., Santos-Mayorga, O. A., Florencio, J. R., Oliveira, M. C., Almeida, L. M. S., Xavier, J. O. L., Zimmermann-Franco, D., Macedo, G., Ferreira, A. L. P., Sousa, O. V., et al. (2023). Vernonia polyanthes Less. (Asteraceae Bercht. & Presl), a Natural Source of Bioactive Compounds with Antibiotic Effect against Multidrug-Resistant Staphylococcus aureus. *Antibiotics*, (12), 622.

[4] Liu, C., Cheng, F., Aisa, H. A., & Maiwulanjiang, M. (2023). Comprehensive Study of Components and Antimicrobial Properties of Essential Oil Extracted from Carum carvi L. Seeds. *Antibiotics*. (12), 591.

[5] Ma, M., Tao, L., Li, X., Liang, Y., Li, J., Wang, H., Jiang, H., Dong, J., Han, D., & Du, T. (2022). Changes in molecular characteristics and antimicrobial resistance of invasive

Staphylococcus aureus infection strains isolated from children in Kunming, China during the COVID-19 epidemic. *Front*. *Microbiol*, (13), 944078.

[6] Wang, M., Buist, G., & Maarten van Dijl, J. (2022). Staphylococcus aureus cell wall maintenance – the multifaceted roles of peptidoglycan hydrolases in bacterial growth, fitness, and virulence. *FEMS Microbiology Reviews,* 46(5), 1-19.

[7] Haag, A. F., Fitzgerald, J. R., & Penadés, J. R. 2019. Staphylococcus aureus in animals. *Microbiol Spectrum,* 7(3), 60.

[8] Linz, M. S., Mattappallil, A., Finkel, D., & Parker, D. (2023). Clinical Impact of Staphylococcus aureus Skin and Soft Tissue Infections. *Antibiotics*, (12), 557.

[9] Raineri, E. J. M., Maas, S., Wang, M., Brushett, S., Medina, L. M. P., Escandell, N. S., Altulea, D., Raangs, E., de Jong, A., Murguia, E. V., Feil, E. J., Friedrich, A. W., Buist, G., Becher, D., Garcia-Cobos, S., Couto, N., & van Dijl, J. M. (2022). Staphylococcus aureus populations from the gut and the blood are not distinguished by virulence traits—a critical role of host barrier integrity. *Microbiome*, (10), 239.

[10] Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., & Choroszy-Krol, I. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. *Gut Pathog,* (11), 10.

[11] Antunes, A., Joao, A. L., Nunes, T., & Henriques, A. R. (2023). Burden of disease estimation based on Escherichia coli quantification in ready-to-eat meals served in Portuguese institutional canteens. *LWT - Food Science and Technology*, (174), 114450.

[12] Daga, A. P., Koga, V. L., Soncini, J. G. M., de Matos, C. M., Perugini, M. R. E., Pelisson, M., Kobayashi, R. K. T., & Vespero, E. C. (2019). Escherichia coli Bloodstream Infections in Patients at a University Hospital: Virulence Factors and Clinical Characteristics. *Front. Cell. Infect. Microbiol,* (9), 191.

[13] Ebenezer, F. N., & Pallen, M. J. (2022). The microbial ecology of Escherichia coli in the vertebrate gut. *FEMS Microbiology Reviews*, 46(3), 1–22.

[14] Bonten, M., Johnson, J. R., van den Biggelaar, A. H. J., Georgalis, L., Geurtsen, J., de Palaciosm, P. I., Gravenstein, S., Verstraeten, T., Hermans, P., & Poolman, J. T. (2019). Epidemiology of Escherichia coli Bacteremia: A Systematic Literature Review. *Clinical Infectious Diseases*, (72).

[15] Pokharel, P., Dhakal, S., & Dozois, C. M. (2023). The Diversity of Escherichia coli Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *Microorganisms,* (11), 344.

[16] Naghavi, Mohsen. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*, (399), 629–55.

[17] Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine and modern industry, Acta Agriculturae Scandinavica, *Section B — Soil & Plant Science*, 69(6), 546-556.

[18] Hua, L. Y., Ning, Z., & Yuebao, N. (2022). Determination of SOD in black ginger extract and its effect on the liver of rats with type 2 diabetes. *Food Sci. Technol, Campinas*, (42), e115021.

[19] Teng, H., Seuseu, K. T., Lee, W. Y., & Chen, L. (2019). Comparing the effects of microwave radiation on 6-gingerol and 6-shogaol from ginger rhizomes (Zingiber officinale Rosc). *PLoS ONE,* 14(6), e0214893.

[20] Unuofin, J. O., Masuku, N. P., Paimo, O. K., & Lebelo, S. L. (2021). Ginger from Farmyard to Town: Nutritional and Pharmacological Applications. *Front. Pharmacol,* (12), 779352.

[21] Setyawan, A. A., Susidarti, R. A., Purwanto., Windarsih, A., Rahmawati, N., Sholikhah, I. K. M., & Rohman, A. (2022). Review on ginger (*Zingiber officinale* Roscoe): phytochemical composition, biological activities and authentication analysis. *Food Research,* 6(4), 443 – 454.

[22] Anh, N. H., Kim, S. J., Long, N. P., Min, J. U., Yoon, Y. C., Lee, E. G., Kim, M., Kim, T. J., Yang, Y. Y., Son, E. Y., Yoon, S. J., Diem, N. C., Kim, H. M., & Kwon, S. W. (2019). Ginger on Human Health: A Comprehensive Systematic Review of 109 Randomized Controlled Trials. *Nutrients,* (12), 157.

[23] Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive Compounds and Bioactivities of Ginger (Zingiber o_cinale Roscoe). *Foods*, (8), 185.

[24] Claudya, R. P., Sugiaman, H. A., Labiba S.I, Utari M.P, Gunawan D. 2023. The therapeutic effects of ginger extract on gastrointestinal disorders to adults. Science Midwifery. 11:1.

[25] Zhang, S., Kou, X., Zhao, H., Mak, K. K., Balijepalli, M. K., & Pichika, M. R. (2022). Zingiber officinale var. rubrum: Red Ginger's Medicinal Uses. *Molecules,* (27), 775.

[26] Juariah, S., Bakar, F. I. A., Bakar, M. F. A., Endrini, S., Kartini, S., Mohamad, A., & Hanafi, A. F. M. (2023). Antibacterial Activity of Red Ginger (Zingiber officinalevar. rubrum) and Black Turmeric (Curcuma caesia) Extracts as Growth Inhibitors of Klebsiella pneumonia. *Trop J Nat Prod Res*, 7(6), 3658-3665.

[27] Zhang, C., Xie ,Y., Qiu, W., Mei, J., & Xie, J. (2023). Antibacterial and Antibiofilm Efficacy and Mechanism of Ginger (Zingiber officinale) Essential Oil against Shewanella putrefaciens. *Plants*, (12),1720.

[28] Adesola, R. O., Ogbole, E., Itodo, A. E., Salami, O., & Abdulazeez, M. D. (2021). Aueous Extracts of Ginger (Zingiber officinale Roscoe) and Garlic (Allium satium L.) Bulbs: Phytochemical Screening and In vivo Antitrypanosomal Effect. *World News of Natural Sciences*, (37), 135-150.

[29] Tritanti, A., & Pranita, I. (2019). The making of red ginger (zingiber officinale rovb. var. rubra) natural essential oil. *Journal of Physics: Conference Series*, (1273), 012053.

[30] Wahid, R. A. H., Purwaningsih, O., & Pamungkas, P. B. (2023). Phytochemical Profiling and Antioxidant Activities of Red Ginger (Zingiber officinalevar. rubrum) Cultivated Eco-Farming. *Trop J Nat Prod Res*, 7(9), 3968-3973.

[31] Badrunanto, Wahyunia, W. T., Farida, M., Batubara, I., & Yamauchi, K. (2024). Antioxidant components of the three different varieties of Indonesian ginger essential oil: In vitro and computational studies. *Food Chemistry Advances 4*, 100558.

[32] Kamal, G. M., Nazi, N., Sabir, A., Saqib, M., Zhang, X., Jiang, B., Khan, J., Noreen, A., Uddin, J., Murtaza, S. (2023). Yield and Chemical Composition of Ginger Essential Oils as Affected by Inter-Varietal Variation and Drying Treatments of Rhizome. *Separations*, (10), 186.

[33] Indiarto, R., Subroto, E., Angeline, & Selly. (2021) Ginger rhizomes (Zingiber officinale) functionality in food and health perspective: a review. *Food Research,* 5(1), 497 – 505.

[34] Suciyati, S. W., Sukrasno., Kurniati, N. F., & Adnyana, I. K. (2021). Antioxidant and Anti-Inflammatory Activity of Red Ginger (Zingiber officinale Roscoe var. Sunti Val) Essential Oil Distillation Residues. *Egypt. J. Chem,* 64(9), 5031 – 5035.

[35] Kwiecinski, J. M., & Horswill, A. R. (2020). Staphylococcus aureus bloodstream infections: pathogenesis and regulatory mechanisms. *Curr Opin Microbiol*, (53), 51–60.

[36] Chukwuma, I. F., Uchendu, N. O., Asomadu, R. O., Ezeorba, W. F. C., & Ezeorba, T. P. C. (2023). African and Holy Basil - a review of ethnobotany, phytochemistry, and toxicity of their essential oil: Current trends and prospects for antimicrobial/anti-parasitic pharmacology. *Arabian Journal of Chemistry,* 16(7), 104870.