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Article

Antibacterial Activity of Combination of Betel Leaf Extract and Star Fruit Using Hydroextraction Method

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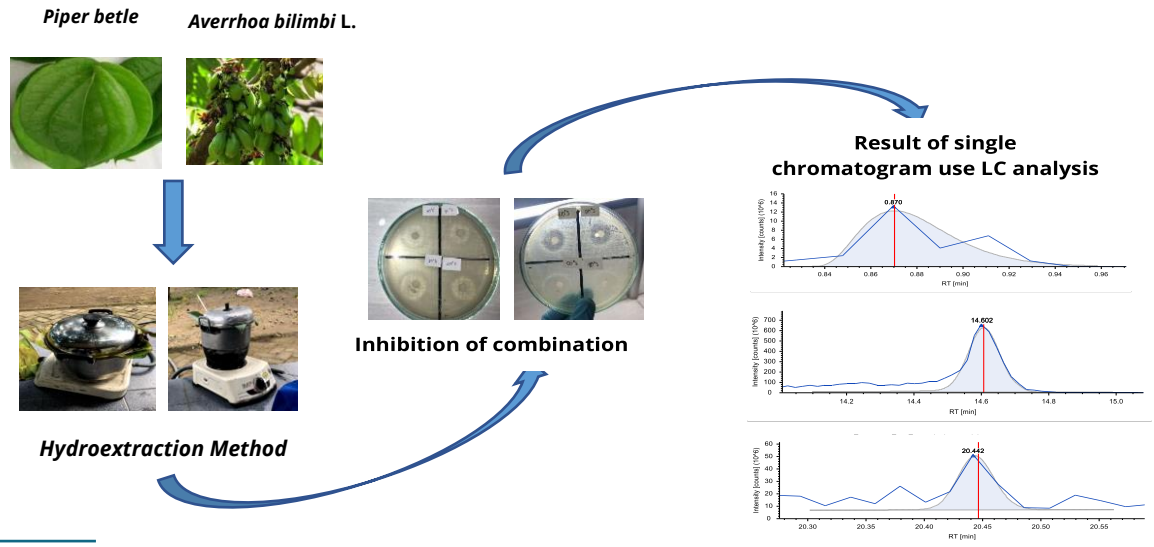
Abstract

Antibacterial compounds are bioactive substances capable of inhibiting bacterial growth by disrupting the metabolism and cellular processes of pathogenic microorganisms. Natural plant-derived compounds have been widely explored for their antibacterial properties, with star fruit (*Averrhoa bilimbi* L.) and betel leaf (*Piper betle*) recognized for their potent antimicrobial effects. This study aimed to evaluate the antibacterial activity of a combination of star fruit and betel leaf extracts against *Escherichia coli* and *Staphylococcus aureus*, two clinically significant bacterial pathogens. The extraction process was performed using hydro-extraction at different temperatures (40°C, 50°C, 60°C, and 90°C) to determine the optimal conditions for bioactive compound yield. Antibacterial activity was assessed using the disc diffusion method, measuring inhibition zones to indicate of bacterial susceptibility. The results demonstrated that the optimal inhibitory effect occurred at 50°C, producing an inhibition zone of 19.75 mm for *Staphylococcus aureus* and 11.75 mm for *Escherichia coli*. These findings suggest that temperature plays a critical role in maximizing the antibacterial potential of plant extracts. The study highlights the potential application of star fruit and betel leaf extracts as natural antibacterial agents, particularly against Gram-positive and Gram-negative bacteria. Further research is recommended to explore the mechanism of action, phytochemical composition, and potential synergy of these extracts in antimicrobial formulations.

Keywords: *Averrhoa bilimbi* L; Hydroextraction, *Piper betle.*

Graphical Abstract



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Introduction

Antibacterial substances are compounds that hinder bacterial growth by interfering with the metabolism of harmful microbes [1]. Antibacterial substances derived from synthetic materials can prevent bacterial infections, but many have side effects such as irritation. This problem encourages the shift in the use of antibacterial substances from synthetic materials to natural materials. Natural material extracts with antibacterial content can be formulated into antiseptic preparations through hand sanitizers or hand soap. Plants with antibacterial properties include starfruit (*Averrhoa bilimbi* L.) and betel leaves (*Piper betle*). These plants are useful as traditional medicines that our ancestors have long used. Starfruit is effective in treating various conditions, diabetes, including coughs, rheumatism, canker sores, mumps, toothaches, acne, bleeding gums, diarrhea, and high blood pressure [2]. Starfruit contains compounds such as saponins, flavonoids, steroids/triterpenoids, and tannins. These compounds exhibit antibacterial activity by inhibiting protein synthesis [3]. According to a study by Aifianti (2014), revealed that starfruit extract displays antibacterial activity against *Staphylococcus aureus and Escherichia coli* bacteria at a 10% concentration. An extract concentration of 10% or the lowest concentration can inhibit bacterial growth and produce an inhibition zone diameter [4]. Meanwhile, betel leaves are efficacious for treating vaginal discharge, eliminating bad breath, treating wounds, stopping bleeding gums, mouth ulcers, and eliminating body odor [5]. The chemical content found in betel plants is saponin, flavonoids, polyphenols, and essential oils [6]. Saponin compounds exhibit antibacterial effects by damaging the cytoplasmic membrane, which results in cell death. Flavonoid compounds, meanwhile, work by causing the denaturation of bacterial cell proteins [7]. Betle leaf extract at a 40% concentration can inhibit *Staphylococcus aureus* bacteria, producing an inhibition zone with a diameter of 17.33 mm [8]. Based on the content of compounds in the two plants that have antibacterial activity, both plants have the potential to be used as natural antiseptics. The combination of these two extracts is anticipated to offer enhanced effectiveness in suppressing bacterial growth. The combination of extracts allows for synergistic activity between compounds.

The extraction technique is the process of transferring a substance or solute from the original solution or solid into a certain solvent [9]. Extraction techniques by maceration, soxhlet, and hydrodistillation can produce very effective antibacterials but require very expensive solvents. Therefore, the hydro extraction method was chosen using hot water by boiling and steaming because this method is considered more economical. Based on this, research is needed to compare the antibacterial activity of a combination of green betel leaf extract and starfruit extract using the hydro-extraction technique. The resulting extract is expected to be more effective and cost-efficient as an antiseptic in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*.

Material and Methods

Materials and Instrumentations

Piper betle and Averrhoa bilimbi extracts, test bacteria *Staphylococcus aureus* and *Escherichia coli*. The test media including Nutrient Agar (NA) and Nutrient Broth (NB). Additional materials are distilled water (H_2O), Mayer's reagent, Dragendorff's reagent, concentrated hydrochloric acid (HCl P), Iron (III) Chloride ($FeCl_3$), ethyl acetate ($C_4H_8O_2$), anhydrous acetic acid, concentrated sulfuric acid (H_2SO_4), hot water, hydrochloric acid (HCl). Other tools are knives, trays, cloths, stoves, pans, beaker glasses, thermometers, petri dishes, measuring cups, droppers, test tubes, test tube racks, analytical scales, spirit lamps, dropper plates, erlenmeyer flasks

Methods

Sample Preparation and Extraction. The plant materials used in this study consisted of green betle leaves (*Piper betle*) and starfruit (*Averrhoa bilimbi* L.), sourced from Bendiljati Wetan Village, Tulungagung, and identified at Materia Medica Batu, Malang. For extract preparation, several fresh yellow starfruits and green betel leaves were selected, washed with clean water, and then allowed to dry at room temperature. Once dried, the samples were cut into small pieces of

approximately 0.5 cm and weighed a total of 100 g. The hydro extraction method was employed, which involved boiling at temperatures of 40°C, 50°C, and 60°C, as well as steaming at 90°C [10]. For the boiling method, 100 ml of water is prepared and heated in a water bath, with temperature settings of 40°C, 50°C, and 60°C. Then, 100 g of the cut samples are added to the heated water and left for 30 min. Afterwards, the mixture is filtered using plastic gauze for the first filtration, followed by a second filtration using filter paper. Treatment with the steaming method, prepare enough water in a steamer that has been perforated on the cover and insert a thermometer in the perforated part for temperature control, insert 100 g of sample, heat on the stove at a temperature of 90ºC, wait approximately 30 min until the extract is obtained then filtered with filter paper [11].

Phytochemical screening. Flavonoids: One gram of the sample extract is placed in a test tube, followed by the addition of concentrated HCl and heating in a water bath for 15 minu. The formation of a red or yellow color indicates a positive result for flavonoids, including flavones, chalcones, and aurones [12]. Tannins: Two grams of the extract are dissolved in ethanol until fully submerged. Subsequently, 1 mL of the solution is transferred into a test tube, and 2–3 drops of 1% $FeCl_3$ solution are added. The presence of tannins is confirmed by the appearance of a bluish-black or green color [13]. Alkaloids: The identification of alkaloid compounds is conducted using Mayer's reagent and Dragendorff's reagent. Terpenoids: Two grams of the sample extract are introduced into a test tube, followed by the addition of 2 mL of ethyl acetate, then shaken. The ethyl acetate layer is separated, transferred onto a dropper plate, and left to dry. Once dried, 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid are added. The formation of a red or yellow color indicates a positive result for terpenoids, whereas a green color signifies the presence of steroids [12]. Saponin: Half a gram of the extracted sample is mixed with 0.5 mL of hot water and shaken for 1 minute. If foam appears, 1N HCl is added. The extract is considered positive for saponins if the foam remains stable for 10 min at a height of 1–3 cm. [14].

Extract Analysis using Liquid Chromatography – High Resolution Mass Spectrometry (LC-HRMS). The combination extract of green betel leaf (*Piper betle*) and starfruit (*Averrhoa bilimbi* L.) was analyzed using a Liquid Chromatography system. The solvents used were A = 0.1% Formic Acid in Water and B = 0.1% Formic Acid in Acetonitrile. The analytical column employed was Hypersil GOLD aQ, with dimensions of 50 x 1 mm and a particle size of 1.9 µm. The analytical flow rate was set at 40 µL/min, and the flow gradient followed the pattern shown in Table 1. The operating time was 30 min, with the column oven temperature maintained at 30°C. HRMS analysis was conducted with a full scan at a resolution of 70,000, with MS2 data captured at a resolution of 17,500. The total running time was 30 min, and the analysis was performed in both positive and/or negative polarity.

Table 1. Gradient Elution

No	Time	Flow Rate (µL/min)	%B	Curve
1	0.000	Equilibration		
2	0.000	40.000	5.0	5
3	*New Row*			
4	0.000	Run		
5	2.000	40.000	5.0	5
6	15.000	40.000	60.0	5
7	22.000	40.000	95.0	5
8	25.000	40.000	95.0	5
9	25.100	40.000	5.0	5
10	30.000	40.000	5.0	5
11	*New Row*			
12	30.000	Stop Run		

Antibacterial Activity. The antibacterial test begins with the preparation of nutrient agar media. To prepare the media, 2 g of agar powder are weighed and dissolved in 100 mL of distilled water. The solution is stirred until fully homogeneous and then sterilized in an autoclave at 121°C for 15 min. After sterilization, extract comparisons between starfruit and green betle leaves are made at a ratio of 20:10 mg/mL, with

chloramphenicol serving as the positive control and distilled water as the negative control. Next, *S. aureus* and *E. coli* bacteria are evenly inoculated on each agar plate. After inoculation, 10 µL of the extract and 10 µL of antibiotics are applied to the designated disc paper on the plates. After the installation of the disc paper is complete, the bacteria are incubated at 37°C for 24 hr, then the diameter of the inhibition zone is observed and measured. The diameter of the inhibition zone is measured using a calliper, and the results are then compared with the positive controls, negative controls, and classification values of the inhibition zone diameter.

Results and Discussions

The sample was extracted using distilled water because distilled water is neutral, has no antibacterial activity, is not easily evaporated and is polar [15]. The method used is the hydroextraction method because this method is more effective and efficient so that it can save production costs. In addition, this method is also easier to apply by the community [10].

Table 2. Secondary metabolites of Combination Extract

Secondary Metabolite	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Steroids	-
Terpenoids	-

Note: (+) contains coumpounds; (-) does not contain compounds

The phytochemical screening test of a combination of green betel leaf extract and star fruit in a 2:1 ratio was conducted by observing the color changes produced by phytochemical reagents in the test tube, which indicated the presence of specific compounds in the extract. The results showed that the combination contained flavonoids, alkaloids, and tannins (Table 1). These findings suggest that the combination may possess potential medicinal and antioxidant properties, whereas saponins, steroids, and terpenoids were not detected.

Profiling Liquid Chromatography – High Resolution Mass Spectrometry (LC-HRMS)

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis revealed that the combination extract of green betel leaf (*Piper betle* L.) and star fruit (*Averrhoa bilimbi* L.) contains bioactive compounds with potential antibacterial properties (Table 3). Among the identified compounds, apigenin, a flavonoid, was detected at a retention time of 0.870 min, with a molecular weight of 271.05991 g/mol (Table 3).

Flavonoids, including apigenin, are known for their antibacterial activity, primarily through interactions with extracellular and soluble proteins, leading to bacterial cell membrane disruption and subsequent leakage of intracellular components [16]. Specifically, apigenin has been reported to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*, two clinically significant bacterial [16-17]. The antibacterial mechanism of apigenin is linked to its ability to inhibit the glucosyltransferase enzyme, which plays a critical role in bacterial adhesion and biofilm formation, thereby reducing bacterial colonization and persistence [18].

These findings align with previous studies highlighting flavonoids as promising natural antibacterial agents that could serve as alternatives or complementary treatments to conventional antibiotics, particularly in addressing antibiotic-resistant bacterial strains [19]. The presence of apigenin in the combination extract of *Piper betle* L. and *Averrhoa bilimbi* L. further underscores the therapeutic potential of plant-based bioactive compounds in the development of novel antibacterial formulations.

Table 3. Antibacterial Active Compounds in Combination Extracts

Name	Formula	Molecular Weight (g/mol)	Retention Time (min)	Area (max)	m/z
Apigenin	$C_{15}H_{10}O_5$	271.05991	0.870	35,328,318.48	99.2
Piperine	$C_{17}H_{19}NO_3$	286.14337	14.602	4,570,066,672.75	98.7
Betaine	$C_5H_{11}NO_2$	118.0863	20.442	101,427,056.68	98.2

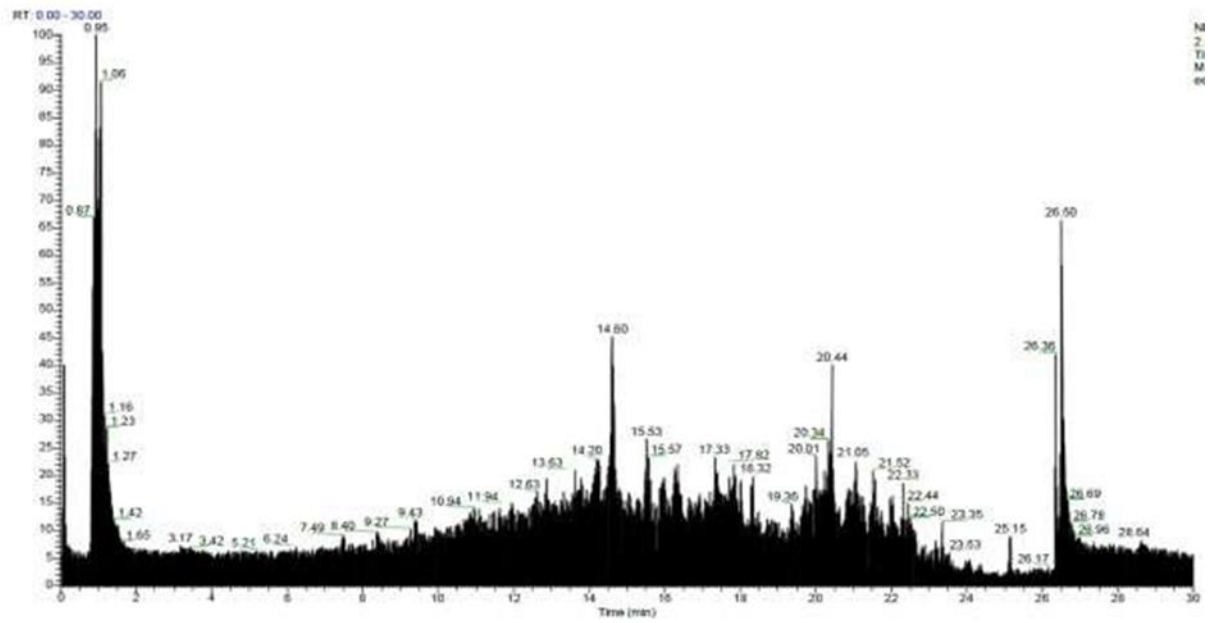


Figure 1. Total Ion Chromatogram

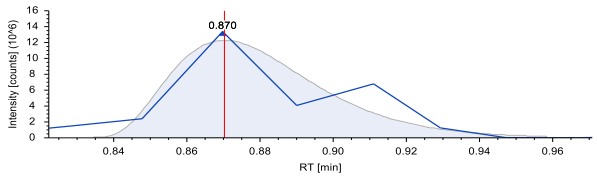


Figure 2. Single chromatogram of Apigenin from LC analysis

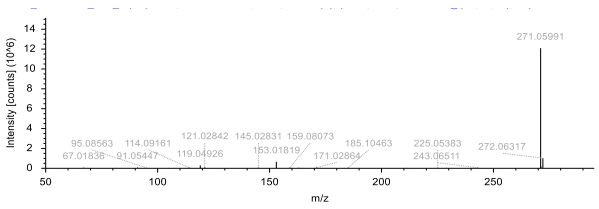


Figure 3, Apigenin Mass Spectrum

The antibacterial mechanism of piperine against *Staphylococcus aureus* is as a protein A inhibitor. Protein A is a protein that is only found on the surface of Staphylococcus aureus bacterial cells [20].

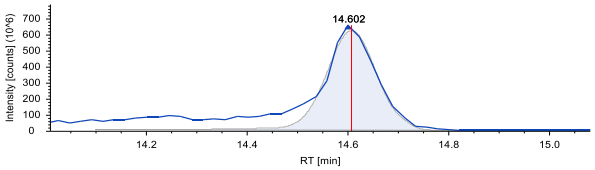


Figure 4. Single chromatogram of Piperine from LC analysis

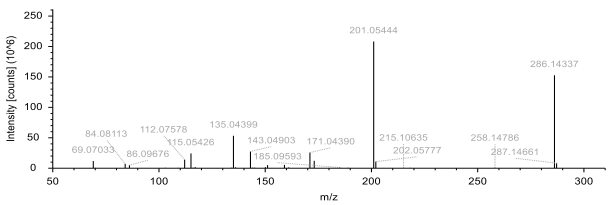


Figure 5. Piperine Mass Spectrum

Betaine compound in the combination extract of betel leaf and starfruit can be eluted at a retention time of 20.442 min (Figure 6) with a molecular weight of 118.0863 (Figure 6). Betaine compound is included in the Tannin compound group. The antibacterial mechanism of tannin has an antibacterial effect by implementing inhibition of protein synthesis. The antibacterial effect of tannins occurs through interactions with cell membranes, enzyme inactivation, and the disruption of genetic material. The mechanism by which tannins act as antibacterials involves inhibiting the reverse transcription enzyme and DNA topoisomerase, preventing bacterial cell formation [16].

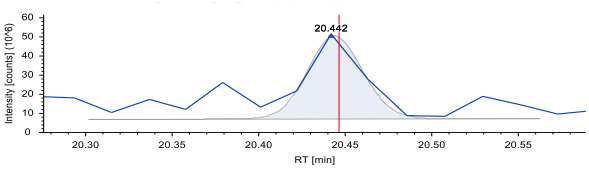


Figure 6. Single chromatogram of Betaine from LC analysis

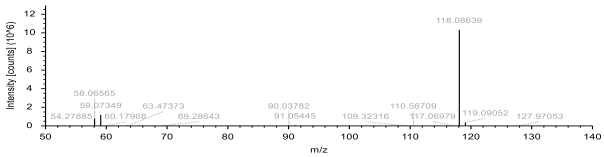
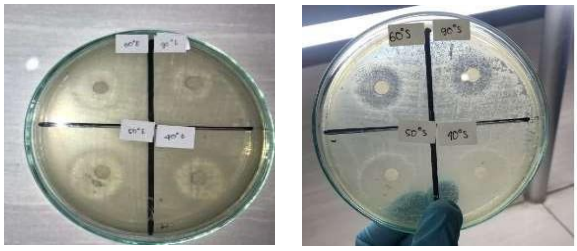


Figure 7. Betaine Mass Spectrum

Antibacterial Test of Combination of Green Betel Leaf Extract and Starfruit Fruit

The antibacterial activity of the combined green betle leaf and starfruit extracts was evaluated using the disc diffusion method. The test plates were incubated at 37°C for 24 hr. To assess the optimum inhibitory effect, the diameter of the clear zone surrounding the disc was measured using a caliper. The results, illustrating the formation of inhibition zones around the disc due to the extract combination, are shown in Figure 8



A B

Figure 8. Inhibition of combination of betle leaf extract and starfruit. (A) *Staphylococcus aureus*, (B) *Escherichia coli*.

Table 4. Diameter of inhibition zone against *Staphylococcus aureus* and *Escherichia coli*

Treatment	***Diameter Clear Zone* (mm)**							
	S. aureus				***E. coli***			
	R1	**R2**	**R3**	**Mean**	**R1**	**R2**	**R3**	**Mean**
40°C	5	24.5	14.75	14.75	8	10	9	9
50°C	17	22.5	19.75	19.75	13	9	11	11
60°C	13	19.5	16.25	16.25	15.5	8	11.75	11.75
90°C	8.5	20.5	14.5	14.4	8	8	8	8
K+	27.5	25.5	35.5	29.5	33	5.5	28	22.17
K-	0	0	0	0	0	0	0	0

R= replication; K+= Positive control (Kloramfenikol); K- = Negative control (aquades)

The results of the antibacterial activity test of the combined extract demonstrated optimal effects at 50°C, producing an inhibition zone of 19.75 mm against Staphylococcus aureus, indicating a strong antibacterial response. Additionally, at 60°C, the extract exhibited optimal activity against Escherichia coli, forming an inhibition

zone of 11.75 mm, which signifies a moderate inhibitory effect on bacterial growth. (Table 4).

The phytochemical screening of the starfruit and betel leaf extract combination confirmed the presence of alkaloids and tannins. Alkaloids are bioactive compounds that act as antibacterial compounds just like bioactive phenol, flavonoid, and tannin compounds. The mechanism is by damaging cell metabolism so that bacterial growth is inhibited.

Natural plant-derived compounds, particularly alkaloids and tannins, have been widely studied for their potent antibacterial properties. Alkaloids exert their antibacterial effects primarily by disrupting the peptidoglycan **structure** of bacterial cell walls, an essential component for maintaining cell integrity and shape. By interfering with peptidoglycan synthesis, alkaloids hinder proper cell wall formation, leading to increased cell permeability, osmotic instability, and ultimately bacterial cell death. In addition to targeting the cell wall, some alkaloids have been reported to interfere with bacterial nucleic acid synthesis and inhibit key metabolic enzymes, further contributing to their bactericidal activity [20].

Similarly, tannins play a significant role in antibacterial defense by inhibiting essential bacterial enzymes, such as reverse transcriptase and DNA topoisomerase, which are critical for bacterial replication and transcription [21]. By blocking DNA synthesis and replication, tannins effectively prevent bacterial growth and proliferation. Moreover, tannins interact with bacterial cell membranes, leading to protein precipitation, enzyme inactivation, and disruption of nutrient transport systems, all of which contribute to bacterial cell death [22].

These antibacterial mechanisms highlight the therapeutic potential of alkaloids and tannins as natural antimicrobial agents, particularly in the fight against antibiotic-resistant bacteria. Recent studies suggest that combining alkaloids and tannins with conventional antibiotics may enhance antimicrobial efficacy, providing a promising strategy to combat multidrug-resistant bacterial infections. As research in phytochemistry and pharmacology advances, alkaloid- and tannin-rich plant extracts continue to be explored as potential alternatives or adjuvants to conventional antibacterial treatments [23-24]. Tannins interact with polypeptides in the bacterial cell wall, disrupting its proper formation. This structural instability makes bacteria susceptible to lysis due to osmotic or physical pressure, ultimately leading to cell death [4].

The study conducted indicates the combination of starfruit extract and betel leaf effectively inhibits the growth of Staphylococcus aureus in the strong category and Escherichia coli in the moderate category. However, the inhibition zone diameter for both bacteria remains smaller than that of the positive control. Staphylococcus aureus is classified as a gram-positive bacterium, whereas Escherichia coli is gram negative. The cell wall of gram-positive bacteria contains a higher concentration of peptidoglycan and fewer lipids, along with polysaccharides. Peptidoglycan is composed of amino acids and sugars, while teichoic acid, a water-soluble polymer, facilitates the transport of positive ions. The polar nature of the gram-positive bacterial cell wall suggests that it is more permeable to polar compounds. Since alkaloids and tannins are polar, they can more readily penetrate the polar peptidoglycan layer compared to the non-polar lipid layer found in gram negative bacteria. This leads to a stronger inhibitory effect on gram positive bacteria than on gram negative bacteria [22].

Conclusion

The analysis of active compounds in the combination extract confirmed the presence of alkaloids and tannins. Antibacterial testing demonstrated that Staphylococcus aureus exhibited optimal inhibition at 50°C, with an inhibition zone measuring 19.75 mm, indicating a strong antibacterial effect. Meanwhile, Escherichia coli showed peak activity at 60°C, forming an inhibition zone of 11.75 mm, which suggests a moderate antibacterial response. Further studies are recommended to explore the antibacterial properties of this extract combination through in vivo testing.

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Author Contributions

Conceptualization, DPT and AM.; Methodology, WH, PIP, and MLK; Software, DPT, WH, and AM.; Validation: DPT and AM; Formal Analysis, DPT and AM.; Investigation, WH, PIP, and MLK; Resources, DPT and AM.; Data Curation, AM and AM; Writing – Original Draft Preparation, DPT, AM, and ILT; Writing – Review & Editing, DPT, AM and ILT and ML; Visualization: DPT and AM.; Supervision, DPT and AM; Project Administration, DPT.

Conflic of Interest

There are no significant conflicts that interfere with the progress of this research, from data collection to processing results and conclusions.

References

[1] Rustanti, E., Jannah, A., & Fasya, A. G. (2013). Uji Aktivitas Antibakteri Senyawa Katekin Dari Daun Teh (*Cameliasinensis* L.Var Assamica) Terhadap Bakteri Micrococcusluteus. *Alchemy*, *2*(2). https://doi.org/10.18860/al.v0i0.2886

[2] Maryam, S., Juniasti, S., & Kosman, R. (2015). Uji Aktivitas Antibakteri Ekstrak Etanol Buah Belimbing Wuluh (Averrhoa Bilimbi L.) Asal Kota Watampone. *Jurnal Ilmiah As-Syifaa*, *7*(1), 60–69. https://doi.org/10.33096/jifa.v7i1.21

[3] Ferdyani, S., Yuniarto, P. F., (2020). Uji Aktivitas Antibakteri Sediaan Gel Ekstrak Etanol Buah Belimbing Wuluh (*Averrhoa Bilimbi* Linn) terhadap Bakteri *Staphylococcus aureus*. *Jurnal Kesehatan Mahasiswa UNIK*, *2*(1), 30–42.

[4] Ngajow, M., Abidjulu, J., & Kamu, V. S. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (*Pometia pinnata*) terhadap Bakteri Staphylococcus aureus secara In vitro. Jurnal MIPA, 2(2), 128. https://doi.org/10.35799/jm.2.2.2013.3121

[5] Dewi, L.K., Sarosa, A.H., Kartikowati, C.W., Hayati, N., Parasu, R., Amalia, E. (2021). Pengaruh Jenis Pelarut Terhadap Daya Antibakteri Hasil Ekstraksi Daun Sirih Hijau (*Piper betle* L.) pada Aktivitas *Staphylococcus Epidermidis*. Journal of Innovation and Applied Technology. Vol. 7 (1). OI: http://dx.doi.org/10.21776/ub.jiat.2021.007.01.6

[6] Noventi, W. R.-4272-2-P. pdfa., & Carolia, N. (2016). Potensi Ekstrak Daun Sirih Hijau (*Piper betle* L .) sebagai Alternatif Terapi Acne vulgaris. Studi Pendidikan Dokter Fakultas Kedokteran Universitas Lampung, Vol. 5(1), Hal. 140.

[7] Triyani, M. A., Pengestuti, D., Khotijah, S. L., Susilaningrum, D. F., & Ujilestari, T. (2021). Aktivitas Antibakteri Hand Sanitizer Berbahan Ekstrak Daun Sirih dan Ekstrak Jeruk Nipis. NECTAR: Jurnal Pendidikan Biologi, 2(1), 16-23

[8] Bagus, I., Suyasa, O., Bekti, H. S., Rinawati, L. P., & Laksmi, L. P. (2022). Daya Hambat Ekstrak Daun Sirih dan Daun Legundi Terhadap Pertumbuhan Bakteri *Staphylococcus aureus*. *1*(5). The Journal Of Muhammadiyah Medical Laboratory Technologist.

[9] Aji, A., & Bahri, S. (2017). Pengaruh Waktu Ekstraksi Dan Konsentrasi HCl untuk Pembuatan Pektin dari Kulit Jeruk Bali (*Citrus maxima*). *Jurnal Teknologi Kimia Unimal*, *6*(1), 33–44.

[10] Rahayu, N. W. S., Prasetyo, E. N., & Isdiantoni. (2016). Hindroekstraksi Daun Ketapang (*Terminalia catappa* L.) sebagai Pengendali Penyakit Ice-ice pada Budidaya Kappaphycus alvarezii. Jurnal Sains Dan Seni Its, 1–8.

[11] Indrayati, A., Farmasi, F., Buana, U., Karawang, P., & Pudding, S. (2019). Formulasi , Uji Stabilitas Fisik Dan Kompatibilitas Produk Kosmetik Anti-Aging Dalam Sediaan Serum. *Jurnal Ilmiah Farmasi*, *4*, 2.

[11] Muthmainnah. (2017). Skrining Fitokimia Senyawa Metabolit Sekunder Dari Ekstrak Etanol Buah Delima (Punica Granatum L.)

Dengan Metode Uji Warna. *Media Farmasi*, *13*(3), 1576–1580.

[12] Harborne, J.B. (2006). Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan (alih bahasa: Kosasih Padmawinata & Iwang Soediro). Bandung : Penerbit ITB.

[13] Dewi, N. R. K., Kuncoro, H., & Rijai, L. (2015). Potensi Sitotoksik Ekstrak Air Daun Sirih Hitam (Piper sp.). *Jurnal sains dan Kesehatan*, *1*(1), 11-15.

[14] Dewantoro, Z., Widodo, Y.L.A., Ciptaningtyas, R. (2017). Pengaruh Pemberian Ekstrak daun Belimbing Wuluh *(Averrhoa bilimbi* L). Terhadap Pertumbuhan Bakteri *Staphylococcus aureus* secara In Vitro. Jurnal Kedokteran Diponegoro, 6(2); 886-892

[15] Parama, P. W., Sukrama, I. D. M., & Handoko, S. A. (2019). Uji efektifitas antibakteri ekstrak buah jeruk nipis (*Citrus aurantifolia*) terhadap pertumbuhan *Streptococcus mutans* in vitro. Bali dental journal, 3(1), 45-52.

[16] Nuria, M.C., A. Faizatun., dan Sumantri. 2009. Uji Antibakteri Ekstrak Etanol Daun Jarak Pagar (*Jatropha cuircas* L) terhadap Bakteri *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, dan *Salmonella typhi* ATCC 1408. Jurnal Ilmu –Ilmu Pertanian. 5: 26 –37.

[17] Isnarianti, R., Wahyudi, I. A., & Puspita, R. M. (2013). *Muntingia calabura* L leaves extract inhibits glucosyltransferase activity of *Streptococcus mutans*. Journal of Dentistry Indonesia, 20(3), 59-63.

[18] Matilla-Cuenca, L., Gil, C., Cuesta, S. et al. Antibiofilm activity of flavonoids on staphylococcal biofilms through targeting BAP amyloids. Sci Rep 10, 18968 (2020). https://doi.org/10.1038/s41598-020-75929-2

[19] Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. Molecules. 2022 Feb 9;27(4):1149. doi: https://doi.org/10.3390/molecules27041149

[20] Yan Y, Li X, Zhang C, Lv L, Gao B, Li M. Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. Antibiotics (Basel). 2021 Mar 19;10(3):318. doi: https://doi.org/10.3390/antibiotics10030318.

[21] Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021 Sep 27;9(10):2041. doi: https://doi.org/10.3390/microorganisms9102041

[22] Redondo LM, Chacana PA, Dominguez JE, Fernandez Miyakawa ME. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front Microbiol. 2014 Mar 27;5:118. doi: https://doi.org/10.3389/fmicb.2014.00118

[23] Sari, D. R. A. P., Yustiantara, P. S., Paramita, N. L. P. V., & Wirasuta, I. M. A. G. (2014). Uji Aktivitas Antibakteri Ekstrak Etanol Buah Lada Hitam (*Piper nigrum* L.) Terhadap Bakteri Propionibacterium acnes. Jurnal Farmasi Udayana, *3*(2), 279831.

[24] Tarigan, I., Muadifah, A., & Latief, M. (2023). Non-Ribosomal Inhibition of The Antibacterial Compound from Ethanol Extract of *Gnetii gnemon*. Life Science, 12(2), 174-185. https://doi.org/10.15294/lifesci.v12i2.67183