JOSAR, Vol.10 No.2 September 2025
ISSN: 2502-8521 (Print) / 2503-1155 (Online)
DOI: https://doi.org/10.35457/josar.v10i2.4940

Sensitivity Test of Clove Leaf Essential Oil (*Syzygium aromaticum*) Against Heterotrophic Bacteria Isolated from Food Products

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Abstract
*This study aimed to evaluate the antibacterial activity of clove leaf essential oil (*Syzygium aromaticum*) against heterotrophic bacteria isolated from food products. Clove leaves were collected from Malang Regency, Indonesia, and the essential oil was extracted via steam-water distillation. Bacterial isolates were obtained from food samples using serial dilution and pour plate techniques. Antibacterial activity was assessed using the agar diffusion method at three oil concentrations (50%, 75%, and 100%), with Amoxicillin trihydrate as the positive control and sterile distilled water as the negative control. The morphological characteristics of the isolates were documented, and inhibition zones were measured. Results indicated that inhibition strength increased with oil concentration up to 75%, with the largest numerical inhibition observed against isolate K2 at 100% concentration (31.3 mm), approaching the positive control's efficacy. Statistical analyses confirmed that differences among treatments were significant (*$p < 0.001$*), except between 75% and 100% concentrations, suggesting a plateau in activity beyond the minimum inhibitory concentration. The findings highlight clove leaf essential oil's potential as a natural antibacterial agent for food preservation, offering an environmentally sustainable alternative to synthetic antimicrobials. However, in vitro conditions may not fully represent performance in real food matrices, warranting further research on its application, stability, sensory effects, and synergistic combinations with other natural antimicrobials.*

Keywords: antibacterial activity, clove leaf essential oil, food safety, heterotrophic bacteria, *Syzygium aromaticum*

1. INTRODUCTION

Indonesia is known as one of the world's largest producers of spice plants, including clove (*Syzygium aromaticum*). Clove is a high-value commodity that has long

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Citation in APA style: Filayani, M.I, Jana, F.N, Fitriyah. (2025). Sensitivity Test of Clove Leaf Essential Oil (*Syzygium aromaticum*) Against Heterotrophic Bacteria Isolated from Food Products. *JOSAR,* Vol.10. No. (2):99-.

Received: August, 11st 2025
Revised: August, 29st 2025
Published: September, 02st 2025

ISSN: 2502-8521 (Print) / 2503-1155 (Online)
DOI: https://doi.org/10.35457/josar.v10i2.4940

been utilized in various sectors, ranging from the cigarette industry and food production to traditional medicine. In addition to the commonly used flower buds, clove leaves also hold great potential due to their essential oil content, which is rich in bioactive compounds particularly eugenol, which is well-known for its antimicrobial properties (Arhim et al., 2023).

Essential oil extracted from clove leaves has been extensively studied and proven to exhibit antibacterial, antifungal, and antioxidant activities. Various studies have demonstrated that eugenol, the main compound in clove leaf essential oil, can damage bacterial cell membranes and inhibit their growth. Previous research by (Rahmawati et al., 2025) showed that clove leaf essential oil is effective against *Staphylococcus aureus* ATCC 25923, in which eugenol acts as the active compound. Additionally, clove leaf essential oil has been reported to inhibit the growth of *Streptococcus mutans* (Hasanuddin & Salnus, 2020). The presence of these bacteria can serve as an indicator of contamination.

In the context of food safety, the presence of heterotrophic bacteria in food products can serve as an indicator of microbial contamination that may pose health risks. Heterotrophic bacteria include various types that utilize organic matter as an energy source and may originate from the processing environment, water sources, or inadequate sanitation of equipment (Alejandre-Colomo et al., 2020). The isolation and testing of heterotrophic bacteria from food products sold at the canteen of UIN Sayyid Ali Rahmatullah Tulungagung are essential as a form of microbiological quality control for the food consumed by the academic community.

Considering the natural antibacterial potential of clove leaf essential oil and the growing need for safe and environmentally friendly alternatives to preservatives or antimicrobial agents, this study was conducted. The aim of this research is to evaluate the sensitivity of clove leaf essential oil against heterotrophic bacteria isolated from food products sold at the canteen of UIN Sayyid Ali Rahmatullah Tulungagung. The results of this study are expected to contribute to the development of essential oil utilization as a natural antibacterial agent in the fields of agriculture, food, and public health.

2. LITERATURE REVIEW

Clove leaf essential oil

Essential oils, also referred to as volatile or ethereal oils, are natural extracts produced by certain plants. These oils are characterized by their ability to evaporate easily at room temperature (20–23 °C) and typically emit a distinctive aroma that reflects their botanical source(Sari et al., 2020). One of the well-known plants that produce essential oil is clove (*Syzygium aromaticum*), with oil that can be distilled from its leaves (2–3%), stems (6%), and flower buds (21.3%). The yield and quality of clove oil are significantly influenced by the extraction process employed. The chemical composition of clove oil depends on several factors, including the plant variety, geographic origin, distillation method, and analytical technique used. Currently, distillation is the most commonly used technique for essential oil extraction, with three primary types: steam distillation, hydro-distillation, and steam-hydro distillation(Prianto, H., 2013).

Among these, steam distillation generally provides a higher yield compared to hydro-distillation, though it requires a longer processing time. Steam-hydro distillation, which combines the advantages of both methods, is also widely used. More recently,

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advancements in technology have introduced microwave-assisted extraction as a novel method for isolating essential oils. This technique accelerates the extraction process and increases product yield, as microwave energy is absorbed by water molecules, rapidly raising the temperature of both the solvent and intracellular water. The resulting increase in temperature can cause cell rupture and enhance the diffusivity of the oil(Nugraha & Chalim, 2019).

Antibiotics and clove leaf essential oil as alternative antimicrobial agenst

Conventional antibiotics are naturally derived or synthetic small molecules designed to inhibit or kill bacteria by disrupting critical cellular processes such as cell wall synthesis (β-lactams, glycopeptides), protein synthesis (aminoglycosides, macrolides, tetracyclines), nucleic acid synthesis (fluoroquinolones, rifamycins), or metabolic pathways (sulfonamides, folic acid analogues). These drugs have underpinned modern medicine and drastically reduced mortality from infectious diseases over the past century (Elshobary et al., 2025).

However, the effectiveness of antibiotics is increasingly compromised by the rapid emergence of antimicrobial resistance. This arises due to mechanisms such as enzymatic drug inactivation, modification of drug targets, reduced uptake or increased efflux of antibiotics, and horizontal gene transfer among bacteria. As highlighted in global reviews, antimicrobial resistance (AMR) now threatens to render common infections untreatable, elevating risks associated with surgery, neonatal care, and chronic disease management (Chinemerem Nwobodo et al., 2022).

3. METHODS

Materials

The clove leaves used in this study were collected from Sidomulyo Hamlet, Tambakasri Village, Sumberanjing Wetan Subdistrict, Malang Regency. Prior to use, the plant was subjected to taxonomic identification to ensure the accuracy of the sample and to confirm its botanical authenticity. Based on the identification results, the clove plant used in this research belongs to the family *Myrtaceae* and is classified as the species *Syzygium aromaticum*. Other materials used in this study included 70% and 90% ethanol, Nutrient Agar (Merck), methylated spirit, and distilled water.

Extraction of clove leaf essential oil

The extraction process was conducted using the steam-water distillation method. The clove leaves used were dark brown in color and had been sun-dried prior to extraction. The dried leaves were subjected to steam-water distillation by placing them on a perforated tray inside the distillation apparatus, while the bottom chamber was filled with water up to a level just below the tray. The distillation process was carried out for 7 hours at a temperature of approximately 90–100 °C (Arhim et al., 2023).

Isolation of heterotrophic bacteria

The isolation of heterotrophic bacteria was performed using serial dilution and pour plate techniques on Nutrient Agar. Raw vegetable samples were collected from the canteen of UIN Sayyid Ali Rahmatullah Tulungagung. One gram of each sample was weighed and ground using a sterile mortar. The homogenized sample was transferred into a test tube containing 9 mL of sterile distilled water and thoroughly mixed to prepare a 10^{-1} dilution. From this, 1 mL was aseptically pipetted into another test tube

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with 9 mL of sterile distilled water to obtain a 10^{-2} dilution. The serial dilution process was continued to prepare 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. From the final three dilutions (10^{-4}, 10^{-5}, and 10^{-6}), 1 mL of each was aseptically transferred into separate sterile Petri dishes. Sterile, molten Nutrient Agar (NA), cooled to approximately 45 °C, was poured into each dish until the surface was completely covered. The contents were gently mixed using a figure-eight motion, and the Petri dishes were sealed with plastic wrap to prevent contamination. All plates were incubated at 28–30 °C for 24–48 hours to allow for bacterial colony development.

Antibacterial Activity Assay of Clove Leaf Essential Oil

The antibacterial activity of clove leaf essential oil was evaluated using the agar diffusion method. Test bacteria were cultured on Nutrient Agar (NA) and evenly spread across the surface of the medium. Wells were then made in the agar using a sterile borer, and each well was filled with a specific volume of clove leaf essential oil. The plates were incubated at 28–30 °C for 24–48 hours, after which the inhibition zones were measured to assess antibacterial efficacy. The size of the clear zone surrounding each well was recorded in millimeters as an indicator of bacterial growth inhibition. The clove leaf essential oil was prepared in three different concentrations: 50%, 75%, and 100%, by diluting with distilled water. Each concentration was accurately formulated using a standard dilution formula to ensure the desired proportion was achieved. Amoxicillin was used as the positive control, while sterile distilled water served as the negative control.

4. RESULTS

The antibacterial effectiveness of clove leaf essential oil (*Syzygium aromaticum*) against bacteria isolated from food materials (vegetables) was tested to determine its ability, at various concentrations, to inhibit bacterial growth. In this study, three concentrations of the essential oil 50%, 75%, and 100% were used, with amoxicillin trihydrate serving as the positive control and sterile distilled water as the negative control. Observations included the morphological characteristics of bacterial colonies and the measurement of inhibition zone diameters for each treatment.

Table 1. Morphological Characteristics of Bacteria

Isolate Code	Colony Margin	Colony Shape	Colony Elevation	Colony Color
K1	Undulate	Circular	Umbonate	White
K2	Undulate	Irregular	Flat	Whitish yellow
K3	Entire	Circular	Convex	White

Morphological characterization of bacterial colonies was performed to identify distinctive features of each isolate. The observations revealed that isolate K1 exhibited an undulate colony margin, circular shape, umbonate elevation, and white pigmentation. Isolate K2 also displayed an undulate colony margin; however, it possessed an irregular shape, flat elevation, and whitish-yellow pigmentation. In contrast, isolate K3 showed an entire colony margin, circular shape, convex elevation, and white pigmentation. These morphological variations may indicate differences in bacterial species or strains isolated from the food samples.

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Table 2. Inhibition zone observations from the antibacterial activity assay of clove oil (*Syzygium aromaticum*) against bacteria isolated from food.

No.	Isolate Code	Concentration (%)	Inhibition Zone Diameter (mm)			Inhibition Strength
			1	2	3	
1	K1	50%	19.3	19.0	17.3	50%
		75%	22.0	18.6	27.2	75%
		100%	18.0	22.3	28.3	100%
		Positive Control (Amoxicillin trihydrate)	38.3	33.0	37.3	Positive Control (Amoxicillin trihydrate)
		Negative Control (Distilled water)	-	-	-	Negative Control (Distilled water)
2	K2	50%	22.3	13.6	21.3	50%
		75%	23.3	24.3	22.6	75%
		100%	22.0	25.6	31.3	100%
		Positive Control (Amoxicillin trihydrate)	36.0	47.3	46.0	Positive Control (Amoxicillin trihydrate)
		Negative Control (Distilled water)	-	-	-	Negative Control (Distilled water)
3	K3	50%	16.7	18.0	23.0	50%
		75%	23.3	20.3	20.3	75%
		100%	23.3	21.6	26.7	100%
		Positive Control (Amoxicillin trihydrate)	37.0	41.0	38.3	Positive Control (Amoxicillin trihydrate)
		Negative Control (Distilled water)	-	-	-	Negative Control (Distilled water)

Table 2 presents the inhibition zone diameters from the antibacterial activity assay of clove oil (*Syzygium aromaticum*) against bacterial isolates K1, K2, and K3 obtained from food samples. In isolate K1, inhibition zones ranged from 17.3–19.3 mm at 50% concentration (strong), 18.6–27.2 mm at 75% (very strong), and 18.0–28.3 mm at 100% (very strong). For isolate K2, inhibition zones were 13.6–22.3 mm at 50% (strong), 22.6–24.3 mm at 75% (very strong), and 22.0–31.3 mm at 100% (very strong). In isolate K3, inhibition zones measured 16.7–23.0 mm at 50% (strong), 20.3–23.3 mm at 75% (very strong), and 21.6–26.7 mm at 100% (very strong). Across all isolates, the positive control (Amoxicillin trihydrate) produced the largest inhibition zones (33.0–47.3 mm, very strong), whereas the negative control (distilled water) showed no inhibition (weak).

The highest inhibition zone among the clove oil treatments was recorded in isolate K2 at 100% concentration, measuring 31.3 mm (very strong), which approached the

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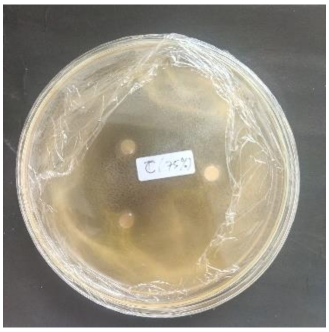
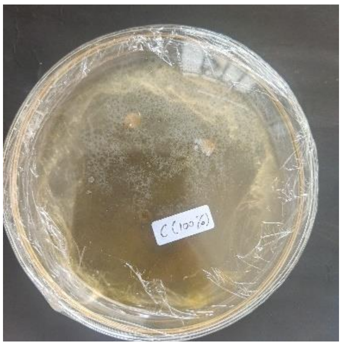
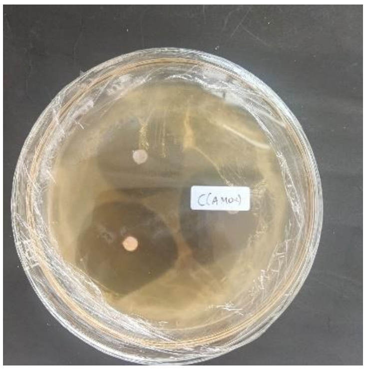
effectiveness of the positive control. This suggests that clove oil at full concentration possesses substantial antibacterial potential, particularly against isolate K2.

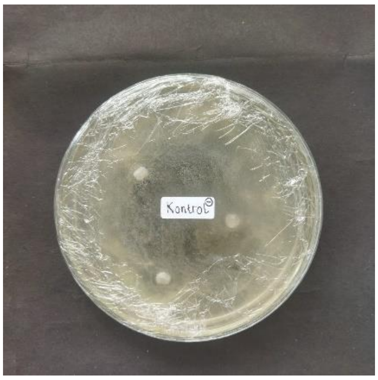
Statistical analysis supported these observations. The Shapiro–Wilk test confirmed that the data were normally distributed ($p > 0.05$ for all treatments), and Levene's test indicated homogeneity of variance ($p > 0.05$). One-way ANOVA revealed a significant difference in inhibition zone diameters among treatments ($p < 0.001$), and the post hoc Duncan test showed that the 75% and 100% concentrations did not differ significantly, but both exhibited significantly greater inhibition than the 50% concentration and the negative control. These results indicate that increasing the concentration of clove oil enhances antibacterial activity up to 75%, with no statistically significant gain beyond that point, although the highest numerical inhibition was observed at 100% against isolate K2.


K1 (50%)

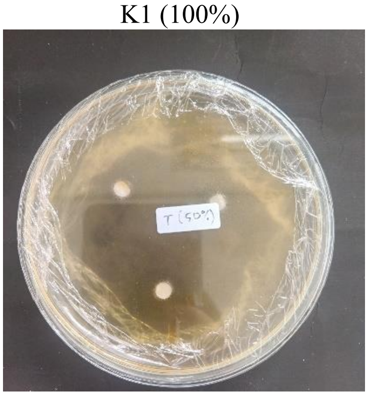

K1 (75%)

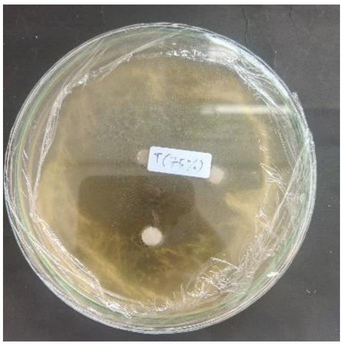

K1 (100%)


K1 (*Amoxicillin trihydrate*)

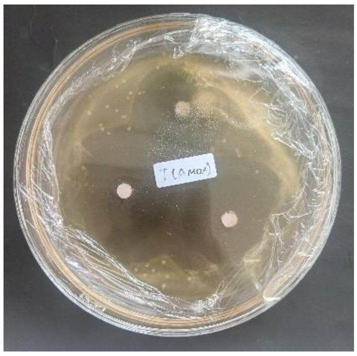


Distilled water


K2 (50%)


K2 (75%)


K2 (100%)


K2 (*Amoxicillin trihydrate*)

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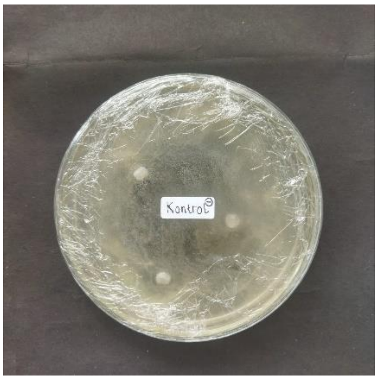
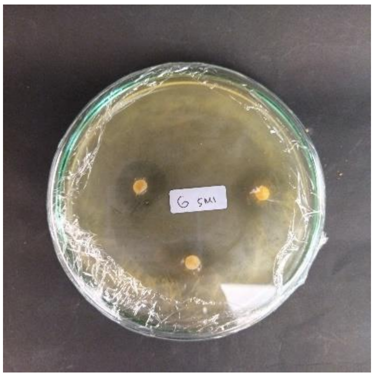
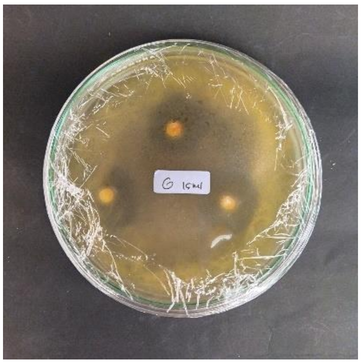
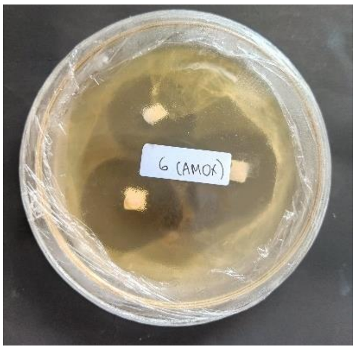

Distilled water


K3 (50%)


K3 (75%)


K3 (100%)


K3
(*Amoxicillin trihydrate*)

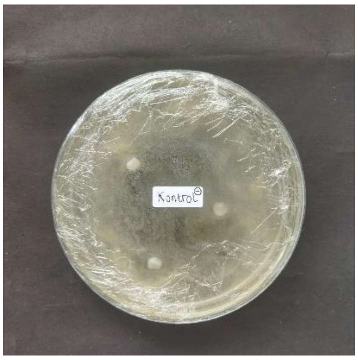

Distilled water

Figure 1. Inhibition zones in the tested bacterial isolates.

5. DISCUSSION

This study confirmed that clove leaf essential oil (*Syzygium aromaticum*) exhibits considerable antibacterial activity against heterotrophic bacteria isolated from food samples, with inhibition strength positively correlated with concentration up to 75%. The highest numerical inhibition was observed for isolate K2 at 100% concentration (31.3 mm), approaching the effectiveness of the positive control (*Amoxicillin trihydrate*). These findings align with previous studies reporting that clove-derived essential oils, particularly those rich in eugenol, possess strong inhibitory effects against a wide range of bacterial species (Hasanuddin & Salnus, 2020; Rahmawati et al., 2025).

The antibacterial efficacy of clove oil can be attributed to the action of eugenol, its primary bioactive constituent. Eugenol is a phenolic compound known to disrupt cytoplasmic membrane integrity, increase membrane permeability, and cause leakage of intracellular constituents, leading to loss of essential cellular functions and cell death (Sari et al., 2020). In addition, eugenol can interfere with enzyme systems involved in energy production and metabolism, further weakening bacterial viability (Elshobary et al., 2025). The complete absence of inhibition in the negative control confirms that the antibacterial activity is attributable to the essential oil itself rather than to the solvent or experimental conditions.

Statistical analysis demonstrated that there was no significant difference in inhibition zone diameter between 75% and 100% concentrations, indicating a potential plateau in antibacterial effectiveness once the minimum inhibitory concentration (MIC) is reached. This plateau effect has been widely documented in essential oil research, where increases beyond the MIC do not significantly enhance inhibition due to saturation of

ISSN: 2502-8521 (Print) / 2503-1155 (Online)
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bacterial target sites (Alejandre-Colomo et al., 2020). Nonetheless, the highest numerical value at 100% in isolate K2 suggests that strain-specific physiological or genetic factors may influence susceptibility, such as variations in membrane composition or efflux pump activity.

Differences in inhibition among isolates K1, K2, and K3 may also be related to differences in cell wall architecture and bacterial taxonomy. Gram-positive bacteria, characterized by a thick peptidoglycan layer, are generally more susceptible to hydrophobic compounds due to easier penetration into the cell wall, whereas Gram-negative bacteria possess an outer membrane that can impede entry of such molecules (Chinemerem Nwobodo et al., 2022). Although Gram staining was not performed in this study, previous research on foodborne heterotrophic bacteria suggests that susceptibility patterns often correlate with Gram classification (Hasanuddin & Salnus, 2020).

From an applied perspective, the observed activity of clove oil against heterotrophic bacteria supports its potential role as a natural antibacterial agent in food preservation. This is particularly relevant in light of growing consumer demand for natural additives and the global need to reduce dependence on synthetic preservatives and antibiotics. The increasing prevalence of antimicrobial resistance (AMR) further underscores the importance of plant-derived antimicrobials as alternative or complementary solutions (Elshobary et al., 2025). Incorporating clove oil into food preservation systems could not only enhance microbial safety but also help slow the spread of resistant strains by reducing selective pressure from conventional antibiotics.

Nevertheless, while the in vitro results are promising, practical application in real food systems requires further evaluation. Factors such as volatility, thermal stability, interactions with food components, and potential effects on sensory quality must be considered (Sari et al., 2020). Future studies should aim to determine the MIC and minimum bactericidal concentration (MBC) of clove oil against a broader spectrum of foodborne pathogens, evaluate its efficacy in complex food matrices, and investigate synergistic effects when combined with other natural antimicrobials. Such research will be essential to bridge the gap between laboratory findings and industrial application, ensuring both safety and consumer acceptability.

6. CONCLUSION

This study demonstrated that clove leaf essential oil (*Syzygium aromaticum*) exhibits strong antibacterial activity against heterotrophic bacteria isolated from food products. The inhibition strength increased with oil concentration up to 75%, with the highest numerical inhibition recorded at 100% against isolate K2 (31.3 mm), approaching the effectiveness of the positive control (Amoxicillin trihydrate). Statistical analysis confirmed significant differences between concentrations, except between 75% and 100%, suggesting a plateau effect in antibacterial efficacy once the minimum inhibitory concentration is reached. These findings highlight the potential of clove leaf essential oil as a natural antibacterial agent for food preservation, offering a sustainable alternative to synthetic preservatives and conventional antibiotics.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided by *the Bantuan Operasional Perguruan Tinggi Negeri* (BOPTN) research grant of 2023,

ISSN: 2502-8521 (Print) / 2503-1155 (Online)
DOI: https://doi.org/10.35457/josar.v10i2.4940

awarded by UIN Sayyid Ali Rahmatullah Tulungagung, Indonesia. This funding played a crucial role in facilitating the successful completion of this research project.

REFERENCES

Alejandre-Colomo, C., Harder, J., Fuchs, B. M., Rosselló-Móra, R., & Amann, R. (2020). High-throughput cultivation of heterotrophic bacteria during a spring phytoplankton bloom in the North Sea. *Systematic and Applied Microbiology*, *43*(2), 126066. https://doi.org/10.1016/j.syapm.2020.126066

Arhim, M., Sukmawati, S., Ariesty Fachrysa Halik, R., & Alim, N. (2023). STRATEGI PENGEMBANGAN USAHATANI MINYAK DAUN CENGKEH (SYZYGIUM AROMATICUM) (Studi Kasus Usaha Industri Minyak Daun Cengkeh di Desa Tallambalao Kecamatan Tammerodo Sendana Kabupaten Majene Provinsi Sulawesi Barat). *WIRATANI : Jurnal Ilmiah Agribisnis*, *6*(1), 2023. http://jurnal.agribisnis.umi.ac.id

Chinemerem Nwobodo, D., Ugwu, M. C., Oliseloke Anie, C., Al-Ouqaili, M. T. S., Chinedu Ikem, J., Victor Chigozie, U., & Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis*, *36*(9), 1–10. https://doi.org/10.1002/jcla.24655

Elshobary, M. E., Badawy, N. K., Ashraf, Y., Zatioun, A. A., Masriya, H. H., Ammar, M. M., Mohamed, N. A., Mourad, S., & Assy, A. M. (2025). Combating Antibiotic Resistance: Mechanisms, Multidrug-Resistant Pathogens, and Novel Therapeutic Approaches: An Updated Review. *Pharmaceuticals*, *18*(3). https://doi.org/10.3390/ph18030402

Hasanuddin, P., & Salnus, S. (2020). Uji Bioaktivitas Minyak Cengkeh (Syzygium aromaticum) Terhadap Pertumbuhan Bakteri Streptococcus mutans Penyebab Karier Gigi. *Bioma: Jurnal Biologi Makassar*, *5*(2), 241–250. http://journal.unhas.ac.id/index.php/bioma

Nugraha, F. Y., & Chalim, A. (2019). Peningkatan Nilai Yield Pada Proses Leaching Jahe Dengan Pelarut Etanol. *DISTILAT: Jurnal Teknologi Separasi*, *5*(2), 206–210. https://doi.org/10.33795/distilat.v5i2.36

Prianto, H., D. (2013). Isolasi dan karakterisasi dari minyak bunga cengkeh (Syzigium aromaticum) Kering Hasil Distilasi Uap. *Kimia Student Journal*, *1*(2), 269–275.

Rahmawati, L. M., Januarti, I. B., Assidiqi, E. R., & Rosyida, R. (2025). Karakteristik dan aktivitas antibakteri minyak atsiri daun cengkeh dari daerah cluwak terhadap bakteri Staphylococcus aureus ATCC 25923. *Journal of Pharmaceutical and Sciences*, 378–383. https://doi.org/10.36490/journal-jps.com.v8i1.484

Sari, N. M., Elsania, F., & Muyassaroh. (2020). Eugenol of clove leaf using microwave steam-hydro distillation with material treatment and power operation variation. *Jurnal Teknik Kimia*, *14*(2), 51–57.