Effect of Extraction Variables on Phenolic Content and Antibacterial Activity of Frangipani (*Plumeria alba*) Leaf Extract by Microwave-Assisted Extraction (MAE)

Sekar Apriana[1], Farikha Maharani[1], Indah Riwayati[1*]
[1]Department of Chemical Engineering, Faculty of Engineering, Universitas Wahid Hasyim
Jl. Raya Manyaran-Gunungpati, Nongkosawit, Kec. Gn. Pati, Kota Semarang, Jawa Tengah 50224
*E-mail: indahriwayati@unwahas.ac.id

Abstract

Antimicrobial resistance is a significant global health issue, prompting the search for new antibacterial sources. This study aimed to evaluate the impact of extraction parameters, such as substrate-to-solvent ratio, extraction time, and microwave power, on the yield and total phenolic content (TPC) of *Plumeria alba L*. leaf extracts using Microwave-Assisted Extraction (MAE) and to assess their antibacterial activity against *Escherichia coli*. Optimization of the extraction process involved varying solvent ratios (1:10, 1:15, 1:20 w/v), extraction times (5, 10, and 15 minutes), and microwave power levels (10%). The results showed that both yield and TPC increased with higher solvent volume and longer extraction times. The optimal conditions (1:20 solvent ratio, 15 minutes extraction time, 10% microwave power) yielded a maximum TPC of 67.98 mg GAE/g, with a significant inhibition zone of 17 mm observed on *E. coli*, indicating strong antibacterial activity. Phenolic and flavonoid compounds play a key role in the bioactivity, exerting effects such as protein denaturation, membrane permeability alteration, and reactive oxygen species (ROS) generation. These findings demonstrate the effectiveness of MAE in extracting bioactive compounds from P. alba leaves and establish a quantitative relationship between extraction parameters, phenolic yield, and antibacterial efficacy. This study provides a foundation for utilizing P. alba leaf biomass as a natural antibacterial agent and promoting the use of green extraction methods in phytochemical research.

Keywords: Frangipani leaves, phenolic, microwave, antibacterial.

INTRODUCTION

Antimicrobial resistance remains a significant challenge to global health, prompting the exploration of novel antibacterial agents from natural sources such as medicinal plants. *Plumeria alba* (white frangipani) leaves contain a variety of secondary metabolites, including flavonoids, phenolic compounds, terpenoids, and essential oils, which exhibit potential antimicrobial and antioxidant properties. While the antibacterial activities of flower and essential oil extracts of P. alba have been demonstrated, limited research has been conducted on leaf extracts, with only one study focusing on a hydro-alcoholic leaf extract (Kaur *et al.*, 2022; Siang *et al.*, 2022).

RBD Palm kernel Oil has potential industrial applications in the soap and food industries. A study utilizing response surface methodology characterized the extraction pattern of RBD palm kernel oil by Microwave-Assisted Extraction (MAE) from palm dates, showing coefficients of

determination (RRM & HADs). MAE is considered a "green" method that enhances efficiency, resulting in higher yields, shorter extraction times, and reduced solvent usage, particularly beneficial for extracting phenolic compounds compared to conventional methods (López-Salazar *et al.*, 2023). This innovative botanical source combined with advanced extraction methods offers a promising avenue for the development of novel antibacterial agents.

Recent investigations have focused on "*Plumeria alba*" and microwave-assisted extraction (MAE) in various areas. A comparative in vitro study demonstrated that the flower extract of P. alba exhibited antimicrobial and cytotoxic effects against oral and periodontal pathogens, highlighting its potential as a natural antibacterial agent (Kaur *et al.*, 2022). Additionally, the P. alba essential oil (EO) displayed strong antioxidant and antimicrobial activities, confirming its bioactive potential and suggesting that different parts of the plant contain pharmacologically important secondary metabolites (Mary Mawumenyo Mamattah *et al.*, 2023).

From a methodological perspective, numerous reports and experimental studies have emphasized that MAE is a cost-effective and sustainable method for extracting bioactive compounds from plant materials. MAE not only improves the extraction efficiency and selectivity of phenolics but also reduces solvent consumption and extraction time compared to traditional maceration or Soxhlet procedures. The success of MAE largely depends on process parameters such as solvent composition, microwave power, temperature, and extraction time (López-Salazar *et al.*, 2023; Alara et al., 2021).

Similarly, an optimization experiment using MAE and response surface methodology (RSM) was conducted by Khalfi et al. (2024) on date seed extraction, focusing on ethanol concentration, temperature, and extraction time to determine the maximum total phenolic content and develop a useful plant extraction model. These findings collectively suggest that applying MAE to *P. alba*, particularly its leaves, holds great potential for increasing antibacterial compound yield and establishing a sustainable and efficient extraction system.

Despite these encouraging developments, there are still key gaps in the literature. While several studies have focused on P. alba flowers and the extracted aromatic oil, research on leaf extracts, particularly regarding their antibacterial properties, is limited. Ethanol extraction of P. alba leaves has shown mild antibacterial and antioxidant properties, even when traditional extraction methods are used instead of modern techniques like Microwave-Assisted Extraction (MAE) (Siang *et al.*, 2022). To date, there has been no systematic study utilizing MAE for *P. alba* leaves or optimizing its key process parameters (such as microwave power, irradiation time, and solvent ratio) to assess their impact on antibacterial activity. Additionally, there is a notable lack of quantitative regression correlation studies linking MAE process conditions to phytochemical yields (total phenolic and flavonoid contents) and antibacterial activities of *P. alba* leaves. This lack of mechanistic correlation hinders the optimization of bioactive effects.

Initial studies on non-optimized macerated leaf extracts have reported negligible antibacterial activity. For example, previous studies on *P. alba* leaf extracts showed no inhibition of Bacillus cereus at certain concentrations, suggesting that the low bioactivity may be attributed to inefficient extraction or suboptimal recovery of active metabolites (Monica *et al.*, 2025). These findings highlight a significant research gap: to date, no study has systematically optimized Microwave-Assisted Extraction (MAE) for *P. alba* leaves and established a quantitative relationship between extraction conditions, chemical profile, and antibacterial efficacy. Addressing this gap could provide a foundation for developing *P. alba* leaf extracts as potential, cost-effective antibacterial remedies.

This study is innovative in several key aspects. Firstly, it utilizes Microwave-Assisted Extraction (MAE) on *Plumeria alba* leaves to optimize extraction parameters such as power irradiation, extraction time, and solvent system to maximize the yields of bioactive compounds, specifically phenolics and flavonoids. Previous research on P. alba has primarily focused on flowers or essential oils, neglecting the potential bioactivity of leaves, which are abundant and likely rich in phytochemicals (Kaur *et al.*, 2022; Mary Mawumenyo Mamattah *et al.*, 2023). Secondly, this study combines an optimized MAE protocol with comprehensive antibacterial assessments (agar diffusion and minimum inhibitory or bactericidal concentration assays) against both Gram-positive and Gram-negative bacteria using leaf extracts instead of floral or oleopathic extracts typically used. Thirdly, the study aims to establish a quantitative correlation between MAE operating conditions and phytochemical composition (total phenolic content; total flavonoid content) through a correlation analysis, linking extraction efficiency variations to biological activity and providing a practical mechanistic explanation of how extraction conditions influence biological potential. While previous research on different plant materials has demonstrated the benefits of MAE in enhancing phenolic extraction and bioactivity (López-Salazar et al., 2023; Khalfi et al., 2024), such relationships have not been explored for P. alba leaves. Lastly, by utilizing leaf biomass, a commonly discarded or undervalued by-product, as the source material, this study presents a sustainable and cost-effective approach to valorise *P. alba* to produce eco-friendly antibacterial agents, aligning with the current trends in green chemistry development and circular bioresource utilization.

The aim of this study was to examine how extraction parameters, such as substrate-to-solvent ratio, extraction time, and microwave power, affect the yield and total phenolic content (TPC) of Microwave-Assisted Extracts (MAE) from *Plumeria alba* leaves. Additionally, the study sought to evaluate the antibacterial potential of these extracts by assessing their inhibitory effects using the agar well diffusion method and measuring the diameter of inhibition zones against selected bacterial strains.

EXPERIMENTAL SECTION

Materials

The main raw material utilized in this study was frangipani (*Plumeria alba*) leaves, with distilled water (aquabidest) used as the solvent for extraction. Analytical-grade gallic acid, sodium carbonate (Na_2CO_3), and Folin–Ciocalteu reagent were employed as standard reagents for determining the total phenolic content. Nutrient Agar (NA) was the growth medium for the antibacterial activity tests.

Instrumentation

The research utilized a Microwave-Assisted Extraction (MAE) unit for extraction and standard laboratory glassware such as Erlenmeyer flasks, beakers, measuring cylinders, round-bottom flasks, and volumetric flasks. Additional tools included a magnetic stirrer, hotplate stirrer, analytical balance, separator, filter paper, and grinder for sample preparation. For microbiological analysis, equipment like a Laminar Air Flow cabinet, autoclave, and inoculating loop were used to maintain sterile conditions. The UV–Vis spectrophotometer was employed for quantitative analysis of the total phenolic content (TPC), while the other instruments supported various stages of the experimental procedure, including preparation, sterilization, and analysis.

Procedure

Preparation

The frangipani (*Plumeria alba*) leaves used in this study were pretreated by drying, grinding, and sieving. The dried leaves were ground using a mechanical grinder and passed through a 100-mesh sieve to obtain a powdered sample (simplicia) with uniform particle size suitable for extraction.

Extraction

Extraction was carried out using Microwave-Assisted Extraction (MAE) with distilled water (aquabidest) as the solvent. To optimize the extraction parameters, two steps were followed:

a) In the initial stage of extraction, the ratio of raw material to solvent was varied at 1:10, 1:15, and 1:20 (w/v) with an extraction time of 5 minutes. The solid residue was filtered, and the filtrate was retained for analysis.

b) In the second step, an optimal extraction time was determined based on the mass to volume ratio established in the first stage. Extraction times were tested at 5 and 10 minutes. The samples were then filtered, and the filtrates were collected for catechins determination.

Preparation of Standard Solutions

Calibration was performed using gallic acid standard solutions at concentrations of 10, 20, 30, 40, and 50 ppm. The absorbance of each concentration was measured using a UV-Vis spectrophotometer, and the data were used to create a standard calibration curve for the quantification of total phenolic content analysis.

Determination of TPC Total phenolic contents (TPC)

In a 10 ml graduated flask, 0.1 g of frangipani leaf extract was diluted with distilled water. One milliliter of each sample solution was transferred to a test tube, followed by the addition of 5 mL of Folin–Ciocalteu reagent. The solution was thoroughly mixed and allowed to stand for 5 minutes. Subsequently, 5 mL of 7.5% Na_2CO_3 was added, and the mixture was incubated in the dark for one hour. The absorbance was measured using a UV–Vis spectrophotometer, and the concentration of total phenolics was calculated in mg gallic acid equivalents (GAE) per gram of extract.

Determination of Extract Yield

To determine the yield, the filtrate obtained from MAE was concentrated using a rotary evaporator until a thick extract was formed. The concentrated extract was accurately weighed using an analytical balance, and the percentage yield was calculated based on the initial weight of the simplicia used.

Antibacterial Inhibition Test

In the antibacterial test, 2 g of NA (nutrient agar) powder was dissolved in 100 mL of distilled water and melted on a hotplate until completely dissolved. The solution was then autoclaved for sterilization. The treated medium was dispensed into Petri dishes and allowed to harden. Each plate was divided into three zones for the extract, positive control, and negative control. The solidified agar surface was inoculated with a bacterial suspension of Escherichia coli using a sterile loop. Sterile paper discs previously impregnated with the frangipani leaf extract, positive control, and negative control were placed on the agar surface. The plates were then incubated at 37 °C, and after 24 hours, the diameters of the inhibition zones around each disc were measured to determine the antibacterial activity of the extract.

RESULTS AND DISCUSSION

Effect of Extraction Conditions on Yield

The impact of the substrate-to-solvent ratio on the yield (%) of *Plumeria alba* leaf extract from Ball-milled Sample Material (BSM) prepared using Microwave-Assisted Extraction (MAE) is illustrated in Figure 1. The yield increased proportionally with the volume of solvent, rising from approximately 10% at a 1:10 ratio to 15% at a 1:15 ratio, and peaking at around 20% at a 1:20 ratio of weight benzoic acid/platinum(IV). This trend indicates that a higher solvent volume enhances the mass transfer process between soluble compounds in the plant matrix and the solvent phase, resulting in more efficient extraction.

A larger solvent volume facilitates greater contact between the solvent and solutes, enabling easier penetration into the plant material and improved diffusion of intracellular contents such as phenolics and flavonoids. This phenomenon has been observed in various studies. For instance, Khalfi et al. (2024) found that increasing the solvent ratio from 1:10 to 1:20 in MAE of date seeds significantly boosted extraction yield and total phenolic recovery by reducing solution viscosity and enhancing microwave energy absorption. Similarly, Chy et al. (2024) noted that optimal extraction of polyphenols from plant materials using MAE required higher solvent volumes to ensure thorough mixing in the matrix and prevent heat-induced artifacts, as uniform temperature distribution over the sample avoids localized heating that could degrade sensitive compounds.

However, as the solvent volume increases proportionally, the concentration of extracted solutes eventually approaches zero, leading to no further gain in extraction efficiency. This observation aligns with the findings of Lopez-Salazar et al. (2023), emphasizing the importance of optimizing solvent ratios to balance solute diffusion and energy efficiency in microwave-assisted extraction (MAE) systems. In this study, a solvent ratio of 1:20 was identified as optimal, ensuring complete wetting and diffusion of all phenolic compounds without excessive dilution.

The increasing yield trend highlights the significant impact of solvent ratio on the efficiency of MAE on P. alba leaves. This finding is consistent with previous studies optimizing MAE on various plant matrices (Khalfi et al., 2024; Chy et al., 2024; López-Salazar et al., 2023) and suggests that the interplay between available solvent and microwave energy distribution plays a crucial role in extraction efficiency.

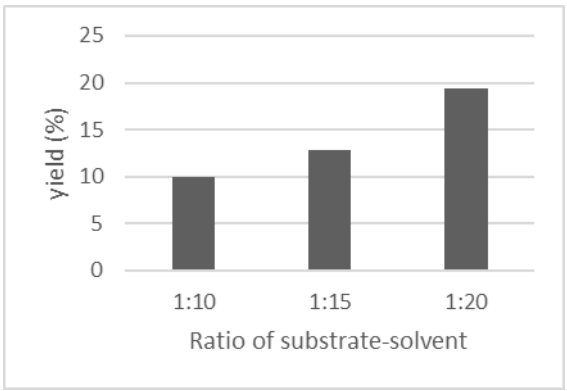

Figure 1. Effect of Solvent Mass-Volume Ratio on Yield

Figure 2 illustrates the impact of extraction time on the yield (%) of *Plumeria alba* leaf extract obtained using Microwave-Assisted Extraction (MAE). The extraction yield increased

with longer extraction times, rising from approximately 20% at 5 minutes to 28% at 10 minutes, and peaking at around 33% at 15 minutes. This trend suggests that extending the extraction time improves solvent penetration and enhances the diffusion of bioactive compounds like phenolics and flavonoids into the solvent phase. According to López-Salazar et al. (2023), prolonged exposure to microwave energy facilitates internal heating and pressure build-up within plant cells, leading to more effective cell wall disruption and solute transfer into the solvent.

The positive relationship between extraction time and yield observed in this study aligns with the results of Khalfi et al. (2024), who noted a significant increase in polyphenol recovery from date seeds with longer MAE durations. Increased extraction time enhances solute-solvent interactions, as reported by Tanruean et al. (2025) in their study on Cinnamomum iners leaves, where a 15-minute MAE process yielded the highest extraction efficiency of phenolic compounds.

Nevertheless, prolonged extraction times can lead to decreased yield due to the degradation or oxidation of heat-sensitive compounds. Mobasheri et al. (2025) demonstrated that excessive microwave irradiation can degrade tannins and flavonoids in pomegranate peel extracts, reducing overall recovery efficiency. This degradation is likely caused by localized overheating and oxidative stress in the solvent medium. Therefore, an optimal extraction duration, such as 15 minutes in this study, strikes a balance between maximizing yield and preventing compound degradation.

Comparable findings were also observed by Febriani et al. (2025) in the MAE of Lansium domesticum (duku) leaves, where the yield increased significantly up to 15 minutes but declined afterward due to phenolic instability from prolonged heating. Additionally, Dao et al. (2022) reported that extending the extraction time within an optimal range enhanced the recovery of catechins from *Camellia sinensis*, while times exceeding 20 minutes caused partial compound degradation due to microwave-induced oxidation.

Overall, the increasing yield observed with prolonged extraction time indicates that microwave irradiation facilitates efficient dielectric heating, promoting solvent penetration, cell wall rupture, and improved mass transfer. Nevertheless, the process must be optimized to avoid excessive thermal stress. The results of this study align with earlier MAE research (Khalfi et al., 2024; Tanruean et al., 2025; Febriani et al., 2025), confirming that extraction time is a critical factor in achieving high yield and process efficiency in plant-based phenolic extraction.

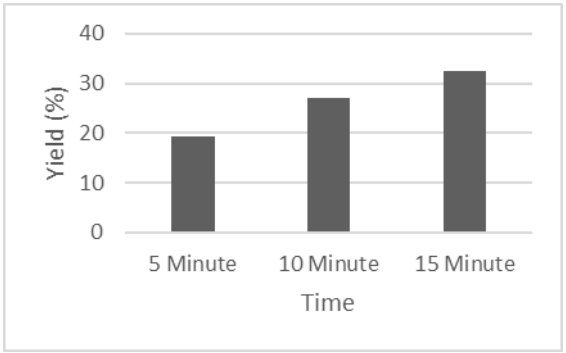

Figure 2. Effect of Extraction Time on Yield

The Effect of Extraction Variables on Total Phenolic Content

Table 1 illustrates the impact of the substrate-to-solvent ratio on the total phenolic content (TPC) of *Plumeria alba* leaf extract obtained through Microwave-Assisted Extraction (MAE) with a

fixed extraction time of 5 minutes and microwave power of 10%. The results show a direct correlation between solvent volume and phenolic recovery. The TPC substantially increased from 12.94 mg GAE/g at a ratio of 1:10 to 35.29 mg GAE/g at 1:15, reaching the highest value of 49.99 mg GAE/g at 1:20.

This increase indicates that higher solvent volumes enhance the solubility and diffusion of phenolic compounds from the plant matrix into the solvent. Larger solvent-to-solid ratios improve mass transfer by reducing viscosity and increasing solvent-solute contact area, resulting in higher extraction efficiency (Khalfi et al., 2024; López-Salazar et al., 2023). Under microwave irradiation, solvent molecules absorb microwave energy more efficiently at higher volumes, leading to uniform heating and faster cell-wall rupture, facilitating the release of bound phenolics. Similar results were observed by Tanruean et al. (2025), who noted a significant improvement in TPC yield when increasing the solvent ratio from 1:10 to 1:20 during MAE of Cinnamomum iners leaves.

At lower ratios (1:10), insufficient solvent limits solute dissolution and can cause localized overheating, reducing phenolic recovery. This pattern was also observed in a study by Febriani et al. (2025) on *Lansium domesticum* leaves, where the phenolic content increased proportionally with the solvent ratio up to 1:20 but plateaued beyond that level. Similarly, Mobasheri et al. (2025) reported that an adequate solvent volume improves microwave penetration and prevents oxidation of phenolics during the extraction of pomegranate peel.

In the current study, the ratio of 1:20 produced the highest phenolic content, indicating that this condition provided sufficient solvent for complete cell hydration and efficient solute diffusion without causing compound degradation. These findings are consistent with previous reports on microwave-assisted extraction (MAE) optimization, highlighting that solvent ratio is a critical parameter influencing total phenolic content (TPC) yield and overall extraction efficiency (Dao et al., 2022; Gavrilă et al., 2025). Therefore, maintaining a balanced solvent volume is essential for maximizing phenolic recovery while preserving compound stability.

Table 1. Effect of Mass-Volume Ratio

Ratio of Substrate-solvent	Extraction Time (min)	IPower (%)	ITotal Phenolic Content (mg GAE/g)
1 : 10	5	10	12,937
1 : 15	5	10	35,287
1 : 20	5	10	49,986

Table 2 illustrates the impact of extraction time on the Total Phenolic Content (TPC) of *Plumeria alba* leaf extract obtained through Microwave-Assisted Extraction (MAE) with a constant solvent ratio of 1:20 and microwave power of 10%. The TPC values exhibited a significant increase from 49.99 mg GAE/g at 5 minutes to 55.86 mg GAE/g at 10 minutes, peaking at 67.98 mg GAE/g at 15 minutes. These results indicate a direct and positive correlation between extraction time and the recovery of phenolic compounds under microwave irradiation.

The rise in TPC with prolonged extraction time is attributed to the enhanced solvent-solute interaction and accelerated cell wall rupture facilitated by microwave heating, allowing for increased diffusion of phenolic compounds into the solvent. Initially, incomplete solvent penetration limits phenolic release, but as time progresses, dielectric heating from microwaves leads to uniform internal heating and localized pressure build-up, effectively disrupting plant

cell walls and releasing bound phenolics (López-Salazar et al., 2023; Khalfi et al., 2024). This mechanism aligns with the findings of Gavrilă et al. (2025), who highlighted the optimal mass transfer achieved with moderate extraction durations (10–20 minutes) in MAE without excessive thermal degradation.

The observed trend is consistent with studies on other plant materials. For example, Tanruean et al. (2025) demonstrated that extending MAE time from 5 to 15 minutes during the extraction of Cinnamomum iners leaves increased TPC due to improved microwave penetration and solvent diffusion. Similarly, Febriani et al. (2025) noted that longer extraction times improved phenolic recovery from *Lansium domesticum* (duku) leaves up to 15 minutes, after which TPC plateaued due to thermal degradation. Mobasheri et al. (2025) also emphasized the importance of optimal microwave exposure for enhancing the extraction of tannins and flavonoids from pomegranate peel, cautioning against overexposure that could lead to oxidative degradation of phenolics.

The strong correlation between extraction time and TPC in this study suggests that phenolic extraction follows diffusion-controlled kinetics, where the rate of extraction is influenced by solvent penetration and compound solubility. Mukhopadhyay et al. (2023) highlighted the role of microwave energy in accelerating solvent motion and enhancing phenolic release compared to conventional methods by providing rapid internal heating of both the solvent and sample. However, prolonged extraction beyond the optimal duration may result in the degradation of sensitive compounds due to extended heating or radical formation, as reported by Patra et al. (2022).

In conclusion, the consistent increase in TPC up to 15 minutes indicates that this duration represents the optimal extraction time for maximizing phenolic recovery from P. alba leaves under MAE conditions. Further extension beyond this time may lead to reduced efficiency and potential compound degradation. These findings are in line with other MAE-based studies (Tanruean et al., 2025; Febriani et al., 2025; Khalfi et al., 2024), emphasizing the importance of optimizing extraction duration to achieve a balance between yield, compound stability, and energy efficiency.

Table 2. Effect of Extraction Time

Rasio of Substrate-solvent	Extraction Time (minute)	Power (%)	Total Phenolic Content (mg GAE/g)
1 : 20	5	10	49,986
1 : 20	10	10	55,860
1 : 20	15	10	67,980

Antibacterial Analysis

The antibacterial test results revealed that the leaf extract of frangipani (*Plumeria alba*) exhibited a significant inhibition zone of 17 mm against Escherichia coli, indicating strong antibacterial activity. According to the diffusion method principle, the size of the inhibition zone reflects the susceptibility of bacteria to antimicrobial compounds, with larger zones indicating higher potency. The 17 mm inhibition zone categorizes the extract as having potent antibacterial activity, based on interpretive criteria for natural antibacterial extracts (Chassagne et al., 2020).

The observed antibacterial effect can be attributed to the presence of phenolic and flavonoid compounds in P. alba leaves, known for their antimicrobial properties. Phenolic compounds

primarily act by denaturing bacterial cell wall proteins and altering membrane permeability. Hydrogen bonding between phenol hydroxyl groups and cellular proteins disrupts enzyme and membrane-bound protein structures, leading to cell membrane damage, cytoplasmic leakage, and ultimately cell lysis (Erikania and Hariningsih, 2017). This mechanism is supported by Kaur et al. (2022), who reported that *P. alba* flower extract inhibits the growth of Staphylococcus aureus and E. coli by disrupting cell membranes and inactivating enzymes through phenolic compounds.

Moreover, the moderate polarity of the extract's solvent system facilitates the efficient extraction of hydroxylated phenolics, enhancing antibacterial efficacy. Studies on similar plant species support these findings: Mamattah et al. (2023) demonstrated that the essential oil and methanolic extract of P. alba showed broad-spectrum antibacterial activity against *Bacillus subtilis* and *E. coli*, attributed to secondary metabolites like plumericin and lupeol. Similarly, Febriani et al. (2025) observed that phenolic-rich extracts from *Lansium domesticum* leaves inhibited *E. coli* with inhibition zones of 15–18 mm, comparable to the current result.

The antibacterial potency observed in this study aligns with other microwave-assisted extraction (MAE) methods, where increased phenolic concentration correlates with enhanced antimicrobial performance. Mobasheri et al. (2025) found that microwave-assisted extracts of pomegranate peel with higher total phenolic content exhibited larger inhibition zones against *E. coli* and *Salmonella typhi*, highlighting the direct relationship between phenolic yield and antibacterial activity. Similarly, Tanruean et al. (2025) demonstrated that polyphenol-rich extracts obtained via MAE showed stronger inhibition against Gram-negative bacteria compared to conventional maceration, attributed to the higher concentration of bioactive compounds.

The antibacterial mechanism of *P. alba* phenolics may involve the generation of reactive oxygen species (ROS) that disrupt bacterial DNA replication and enzyme systems, as reported by Altuntas and Korukluoglu (2024). Gram-negative bacteria like *E. coli* are typically more resistant due to their outer membrane barrier, but certain phenolics, particularly hydroxycinnamic acids and flavones, can penetrate and destabilize this layer, leading to growth inhibition.

Therefore, the 17 mm inhibition zone observed in this study confirms that *Plumeria alba* leaf extract contains bioactive phenolic compounds capable of inhibiting bacterial growth through multiple mechanisms—protein denaturation, membrane disruption, and oxidative stress induction. These findings are consistent with previous literature on the antimicrobial properties of phenolic-rich plant extracts and further support the potential of *P. alba* leaves as a natural antibacterial source suitable for developing herbal antimicrobial agents.

CONCLUSION

This study confirmed that Microwave-Assisted Extraction (MAE) effectively increased the yield and total phenolic content (TPC) of *Plumeria alba* leaf extracts. The optimal extraction conditions, including a 1:20 solvent ratio, 15-minute extraction time, and 10% microwave power, resulted in the highest TPC (67.98 mg GAE/g) and exhibited strong antibacterial activity with a 17 mm inhibition zone against *Escherichia coli*. The antibacterial effect is primarily due to phenolic and flavonoid compounds that disrupt bacterial cell membranes and proteins. Overall, MAE demonstrated to be an efficient and environmentally friendly method for producing bioactive extracts, showcasing *P. alba* leaves as a promising natural antibacterial source.

REFERENCES

Alara, O. R., Abdurahman, N. H. and Ukaegbu, C. I. (2021) 'Extraction of phenolic compounds: A review', *Current Research in Food Science*, 4, pp. 200–214. doi: https://doi.org/10.1016/j.crfs.2021.03.011.

Altuntas, S. and Korukluoglu, M. (2024) 'the Role of Phenolic Compounds As Potential Antimicrobial Agents', *Futuristic Trends in Agriculture Engineering & Food Sciences Volume 3 Book 7*, 3, pp. 503–516. doi: 10.58532/v3bcag7p1ch27.

Chassagne, F. *et al.* (2020) 'A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective.', *Frontiers in pharmacology*, 11, p. 586548. doi: 10.3389/fphar.2020.586548.

Chy, M. W. R. *et al.* (2024) 'Optimization of microwave-assisted polyphenol extraction and antioxidant activity from papaya peel using response surface methodology and artificial neural network', *Applied Food Research*, 4(2), p. 100591. doi: https://doi.org/10.1016/j.afres.2024.100591.

Dao, D. T. A. *et al.* (2022) 'Research on microwave-assisted extraction with blanching pretreatment effect on bioactive compounds of green tea leaves (Camellia sinensis) powder', *Journal of Food Processing and Preservation*, 46(7), p. e16690. doi: https://doi.org/10.1111/jfpp.16690.

Erikania, S. and Hariningsih, Y. (2017) 'Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kamboja (Plumeria Sp) Terhadap Bakteri Escherichia coli Secara In Vitro', *Edu Masda Journal*, 1(1), p. 74. doi: 10.52118/edumasda.v1i1.45.

Febriani, H. *et al.* (2025) 'Optimization of microwave-assisted extraction to obtain a polyphenol-rich crude extract from duku (Lansium domesticum Corr.) leaf and the correlation with antioxidant and cytotoxic activities', *Kuwait Journal of Science*, 52(1), p. 100315. doi: https://doi.org/10.1016/j.kjs.2024.100315.

Gavrila, A. I. *et al.* (2025) 'Optimization of Microwave-Assisted Extraction of Polyphenols from Crataegus monogyna L.', *Antioxidants*. doi: 10.3390/antiox14030357.

Kaur, J. *et al.* (2022) 'Antimicrobial and cytotoxicity properties of Plumeria alba flower extract against oral and periodontal pathogens: A comparative in vitro study.', *Journal of Indian Society of Periodontology*, 26(4), pp. 334–341. doi: 10.4103/jisp.jisp_329_21.

Khalfi, A. *et al.* (2024) 'Optimization of the Microwave-Assisted Extraction Conditions for Phenolic Compounds from Date Seeds.', *Foods (Basel, Switzerland)*, 13(23). doi: 10.3390/foods13233771.

López-Salazar, H. *et al.* (2023) 'Microwave-assisted Extraction of Functional Compounds from Plants: A Review', *BioResources*, pp. 6614–6638. doi: http://dx.doi.org/10.15376/biores.18.3.Lopez-Salazar.

Mary Mawumenyo Mamattah, K. *et al.* (2023) 'Chemical Characterization, Antioxidant, Antimicrobial, and Antibiofilm Activities of Essential Oils of Plumeria alba', *Biochemistry Research International*, 2023(1), p. 1040478. doi: https://doi.org/10.1155/2023/1040478.

Mobasheri, F. *et al.* (2025) 'Machine learning optimization of microwave-assisted extraction of phenolics and tannins from pomegranate peel', *Scientific Reports*, 15(1), p. 19439. doi: 10.1038/s41598-025-04798-4.

Monica, S. *et al.* (2025) 'Azerbaijan Pharmaceutical and Pharmacotherapy Journal Impact Of Ethanol Extract Ratios from Leaves and Flowers of White Frangipani (Plumeria Alba) On the Growth of Bacillus Cereus as A Cause of Food Poisoning', 1951, pp. 1–5. Available at: https://doi.org/10.61336/appjWebsite:https://azpharmjournal.com/en/.

Mukhopadhyay, S., Dutta, R. and Das, P. (2025) 'Process optimization of microwave-assisted extraction (MAE) of polycyclic aromatic hydrocarbons (PAHs) from leaves of Murraya paniculata (L.) Jack: Process intensification approach', *Chemical Engineering and Processing - Process Intensification*, 212, p. 110284. doi: https://doi.org/10.1016/j.cep.2025.110284.

Patra, A., Abdullah, S. and Pradhan, R. C. (2022) 'Review on the extraction of bioactive compounds and characterization of fruit industry by-products.', *Bioresources and bioprocessing*, 9(1), p. 14. doi: 10.1186/s40643-022-00498-3.

Siang, C. C. *et al.* (2022) 'Antioxidant and antibacterial response of hydroalcoholic extract of Plumeria alba leaves', *International journal of health sciences*, 6(April), pp. 12393–12411. doi: 10.53730/ijhs.v6ns2.8279.

Tanruean, K. *et al.* (2025) 'Optimization of Microwave-Assisted Extraction Process for Production of Polyphenol-Rich Crude Extract from Cinnamomum iners Leaves', *Applied Sciences*. doi: 10.3390/app15031265.