

Bioactive Metabolites in *Melaleuca leucadendra* Leaves Extract: Phytochemical Profiling and GC-MS Characterization for Antimicrobial Relevance

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Abstract

The global escalation of antimicrobial resistance (AMR) necessitates the exploration of natural sources of antibacterial agents. This study examined the ethanolic leaf extract of *Melaleuca leucadendra*'s phytochemical composition, chemical profile, and antibacterial activity. Alkaloids, flavonoids, saponins, tannins, phenolics, terpenoids, and steroids were all detected by phytochemical screening. The quantitative total flavonoid content (TFC) of the extract was 17.78 mg QE/g, while the total phenolic content (TPC) was 292.43 mg GAE/g extract. Gas Chromatography–Mass Spectrometry (GC–MS) analysis identified oxygenated esters (18.86%), oxygenated aromatics (15.22%), phenolic derivatives (13.44%), and flavonoids (12.86%), as well as methoxylated aromatics and terpenoids (5.34%), phytol, and fatty acid derivatives. These metabolite classes are widely reported to exert antibacterial effects through multiple mechanisms such as membrane disruption, enzyme inactivation, and inhibition of nucleic acid synthesis. Antibacterial testing against *Staphylococcus aureus* and *Escherichia coli* using the agar diffusion method showed a concentration-dependent response, with the highest activity at 80% extract (15.67 ± 0.58 mm and 15.33 ± 1.15 mm inhibition zones, respectively). Raman spectroscopy confirmed the interaction between polymer molecules and secondary metabolite compounds in the extract, thereby potentially enhancing antibacterial properties. These findings highlight that the antibacterial activity of *M. leucadendra* is mediated by the synergistic interplay of phenolics, flavonoids, methoxylated aromatics, terpenoids, and fatty acids. The findings offer compelling proof that *M. leucadendra* is a viable natural source for the creation of antibacterial compounds that will lessen antibiotic resistance.

Keywords: Antibacterial, bioactive compounds, GC-MS analysis, *Melaleuca leucadendra*

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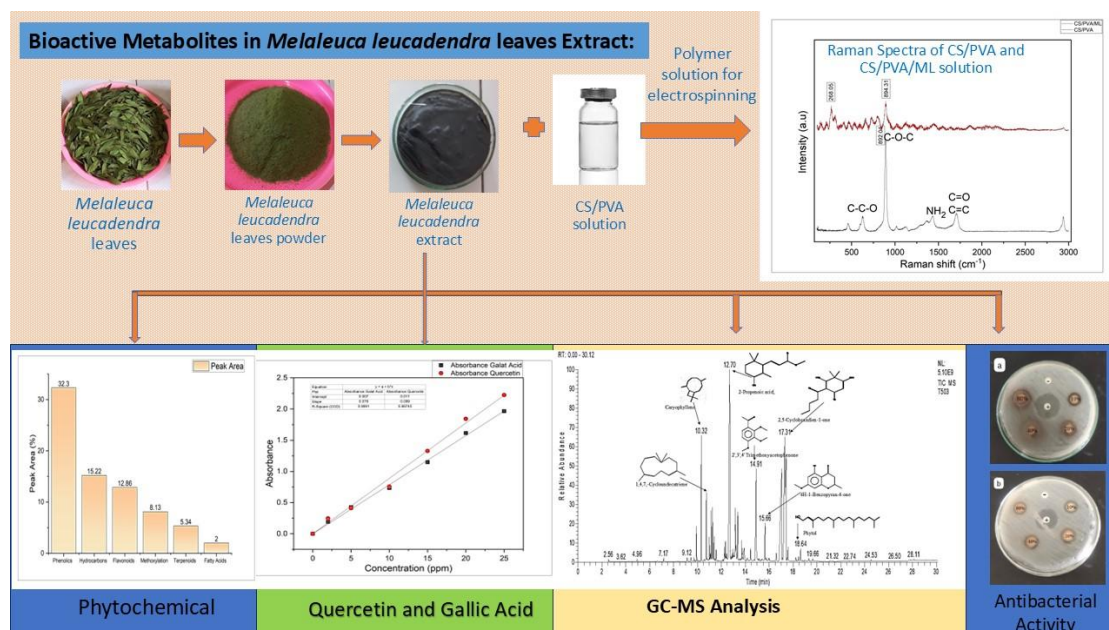
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Graphical Abstract



Introduction

Microbial resistance (AMR) to antibiotics has become one of the greatest challenges in global health [1,2]. Studies show a significant increase in AMR rates in 76 countries during 2000–2015 [3]. This issue is a clinical problem that requires a multidisciplinary approach involving surgery, microbiology, and clinical pharmacy [4]. The World Health Organization (WHO) has classified AMR as a serious threat that could lead to a future health crisis if not addressed immediately [5], with global health implications including increased morbidity, mortality, and healthcare costs [6]. Excessive and inappropriate use of antibiotics is a major factor accelerating the emergence of resistant bacterial strains [7]. Therefore, the search for and development of new antibacterial compounds [8], especially those derived from natural sources, has become an increasingly important research focus.

Medicinal plants are a potential source of bioactive compounds that can be used as alternatives to synthetic antibiotics [9], such

as curcumin integrated into *Phaseolus vulgaris* polysaccharide bio-composite films that can serve as a sustainable matrix for drug delivery systems with wound healing potential [10], *Ricinus communis* (castor bean leaves) structurally optimizes the antiproliferative and pro-apoptotic activity of CuONPs nanoparticles for lung cancer therapy [11]. Through a variety of mechanisms, including compromising the integrity of cell membranes, preventing protein synthesis [12], and deactivating bacterial metabolic enzymes [13], it has been demonstrated that a variety of secondary metabolites produced by plants, including flavonoids, alkaloids, tannins, saponins, and terpenoids, have antibacterial activity [14]. The use of medicinal plants also has advantages in terms of abundant availability, relatively low production costs, and the possibility of minimal side effects compared to synthetic antibiotics.

One medicinal plant with great potential as a source of antibacterial compounds is the gelam leaf (*Melaleuca leucadendra* L.) [15], a member of the *Myrtaceae* family. This plant is widely distributed in tropical regions,

including Indonesia, and is traditionally used to treat infections, inflammation, and various skin diseases. Various studies have reported that *Melaleuca* species contain essential oils [16] with main components such as eucalyptol, α -pinene, and terpinen-4-ol, which have antimicrobial activity [17][18]. Previous studies have shown that ethanol extracts of plants belonging to the *Melaleuca* genus contain secondary metabolites that are not completely soluble in the essential oil fraction, so that polar-semi-polar solvent extraction methods such as ethanol can reveal additional bioactive components that have the potential to increase the spectrum of antibacterial activity.

Most studies have focused on essential oils, while comprehensive research on ethanol extracts of *M. leucadendra* leaves, including phytochemical analysis, compound identification using GC-MS, and antibacterial activity testing, remains limited. This opens up opportunities to discover new candidate compounds that have not been identified in essential oil studies alone. Based on this background, this study aims to: (1) conduct phytochemical screening of *M. leucadendra* leaf ethanol extracts to identify the secondary metabolite groups contained therein, qualitative analysis of total flavonoid content and total phenolic content (2) analyze the compound profile in the crude extract using Gas Chromatography –Mass Spectrometry (GC-MS) instrument, (3) assess the antibacterial activity of gelam leaf ethanol extract against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria using the agar diffusion method, and (4) analyze the potential interaction of extract components with polymers for biomedical use. It is anticipated that this research will aid in the creation of organic plant-based antibacterial substances that effectively combat antibiotic resistance

Materials and Methods

Materials

Fresh *Melaleuca leucadendra* leaves were collected from the Sekojo area, Palembang, Indonesia. 96% ethanol for analysis was purchased from PT. Sumber Kita Indah, Bekasi, Indonesia. All reagents for phytochemical screening, including Mayer's and Dragendorff's reagents (for alkaloids), FeCl_3 solution (for phenolics and tannins), Liebermann–Burchard reagent (for steroids and terpenoids), Mg-HCl reagent (for flavonoids), and aquadest, were of analytical grade. Mueller–Hinton Agar (MHA) was obtained from HIMEDIA M173. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as test bacteria, analytical balance (accuracy ± 0.0001 g), rotary evaporator (Yamato Scientific RE301 series), GC-MS system (Thermo Fisher Scientific ISQ series with Trace 1300), acid cabinet, Petri dishes, measuring cups, Erlenmeyer flasks, paper disks, autoclave (UNICLAVE FD50A), incubator (Equitron Incubator Stream Series), micropipette (10–1000 μL), caliper or ruler (for measuring inhibition zones).

Plants Determination

Gelam (*Melaleuca leucadendra*) was collected from swampy areas in Kalidoni District, Palembang, Indonesia. *Melaleuca leucadendra* was identified by a botanist at the Biology Laboratory of Universitas PGRI Palembang. The official letter of plant determination confirming the species as *Melaleuca leucadendra* was issued under letter number 133/E.12/FST/UPGRIP/2024.

Extraction

The extraction method using maceration followed the procedures [9,19,20] with minor changes. Fresh leaves were washed, dried at room temperature (25–27°C) to a

constant weight, and ground into coarse powder. A total of 500 g of leaf powder was soaked in 96% ethanol at a ratio of 1:10 (w/v) in a closed container at room temperature for 3×24 h, with stirring every 1×24 h. The mixture is filtered through Whatman No. 1 filter paper, and the filtrate is collected. The residue is re-macerated twice under the same conditions to ensure complete extraction. The combined filtrate is then evaporated under low pressure using a rotary evaporator at 60°C to obtain a crude ethanol extract. The crude extract is stored at 4°C until further analysis. The yield is calculated using the following question 1.

$$\text{Yield (\%)} = \frac{\text{Extract mass (g)}}{\text{Crude mass (g)}} \times 100 \% \quad (1)$$

Qualitative Analysis of Phytochemical Compounds

Phytochemical analysis was performed to identify the presence of major secondary metabolites using color reagents [21,22,23]. Alkaloid: Mayer's reagent, the formation of a white precipitate (Mayer) indicates the presence of alkaloids. Flavonoids: magnesium powder and HCl, the appearance of a red/orange color indicates the presence of flavonoids. Saponins: foam test, foam that lasts more than 15 min indicates the presence of saponins. Tannins: 1% FeCl₃ solution, a blue-black or green-black color indicates the presence of tannins. Phenolic: 1% FeCl₃ solution, blue or green color indicates the presence of these compounds. Terpenoid: Liebermann-Burchard reagent, the formation of a reddish-brown color indicates the presence of these compounds. Steroids: Liebermann-Burchard reagent, the appearance of a green/blue color indicates the presence of these compounds. The results are recorded qualitatively as "presence" (+) or "absence" (-).

Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

TFC were determined using the methods described by previous studies [24,25] using the aluminum chloride colorimetric assay, and the absorbance was measured at 415 nm. The Folin-Ciocalteu method was used to evaluate the TPC of the extract as described in [26-28], with absorbance measured at 765 nm.

Chromatography-Mass Spectroscopy (GC-MS) Analysis

The chemical composition of ethanol extracts was determined using Gas Chromatography-Mass Spectroscopy (GC-MS) [29-31], with a qualitative method. GC-MS analysis using Thermo Fisher Scientific ISQ series with trace 1300, TG-5MS column type, flow rate of 1.200 mL/min, and Helium as the carrier gas. **Instrument setup:** The analysis was performed in GC-MS full scan EI (electron ionization) mode, positive ion polarity, mass range m/z 30–800 with a scan time of 0.2 s per scan. The transfer line temperature was 290 °C and ion source temperature was 270 °C. Acquisition began after 2.5 min (segment #1) and was recorded as TIC. Chromatographic filtering was enabled during acquisition. **Oven program (GC):** The oven was initially set to 60 °C (held for 1.0 min), then heated gradually at 10 °C/min to 200 °C (hold for 2.0 min), 220 °C (hold for 2.0 min), 250 °C (hold for 2.0 min), and 270 °C (hold for 2.0 min). Maximum oven temperature 350 °C; cryogenic inactive. GC run time is used as the acquisition duration. **Inlet & injection:** Injection is performed on S/SL splitless (inlet temperature 200 °C), sample volume 1.00 µL. Splitless time 0.50 min, then purge at 5.0 mL/min. Carrier flow mode constant flow 1.200 mL/min; gas saver 10.0 mL/min starting at 2.0 min. Backflush active from 1.50 min until the end of the run. **Analysis**

data: The software used in this study was XCalibur. The mass spectra of the compounds were compared to The National Institute of Standard and Technology (NIST) database in order to determine their identities.

Antibacterial Activities

The antibacterial activities of ethanol extracts were evaluated using the agar diffusion method [32,33,34] against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Each bacterial strain was grown in nutrient medium at 37 °C for 18–24 h, adjusted to McFarland standard 0.5 ($\sim 1.5 \times 10^8$ CFU/mL). Mueller–Hinton Agar (MHA) plates were evenly inoculated with bacterial suspension using sterile cotton swabs. A 6 mm diameter paper disk was placed on top of the MHA containing bacteria, then 10 μ L of ethanol extract at concentrations of 10%, 20%, 40%, and 80% (w/v with ethanol) was dropped onto the disk. Aquadets was used as the negative control due to its neutral and non-antibacterial nature, and 30 μ g of tetracycline as the positive control as a standard antibiotic. The plates were incubated at 37 °C for 24 h, after which the clear zones were measured. Each antibacterial assay was carried out in triplicate ($n = 3$). Standard deviation (SD) values were calculated using the standard deviation formula. Antibacterial power (a) was calculated by comparing the inhibition of the sample with the inhibition zone of the positive control [35], according the equation 2.

$$a = \frac{\text{inhibition zone of the sample}}{\text{inhibition zone of the positive control}} \quad (2)$$

$a < 50\%$ is weak antibacterial activity, moderate antibacterial activity if $50 \leq a \leq 70$, and strong antibacterial activity if $a > 70$ [35].

Raman Spectroscopy

Raman spectroscopy testing was conducted to evaluate the potential of gelam leaf extract as a natural polymer for nanofibers in biomedical applications. Two types of polymer solutions were prepared: chitosan/polyvinyl alcohol (CS/PVA) solution and CS/PVA/ML solution (with 3% gelam leaf extract added). Solution preparation followed the procedure of [36]. Raman spectroscopy was performed at a wavelength of 785 nm on both solutions, and analysis was carried out by identifying peak shifts on the graph.

Results and Discussion

Determination

The identification process was carried out by botanists in the laboratory to verify the authenticity of the materials used in the study [37]. The results showed that the plant belonged to the Myrtaceae family, the *Melaleuca* genus, with the species identified as *Melaleuca leucadendra*. The morphological characteristics of the gelam plant are woody, with peeling grayish-white to red bark, pointed green leaves, round flowers and inflorescences that are usually white, flower clusters with dangling stamens, and roots that tend to be shallow and spread out as an adaptation to wet soil or swamps [38].

Qualitative Analysis of Phytochemical Compounds

The extraction process from 500 grams of dried leaves powder produced 78.03 grams of crude extract with a calculated yield of 15.61%. The extraction process is illustrated in **Figure 1**. 3 liters of maserat were evaporated at a temperature 60°C, and the crude extract was stored at a temperature of 4°C before use.

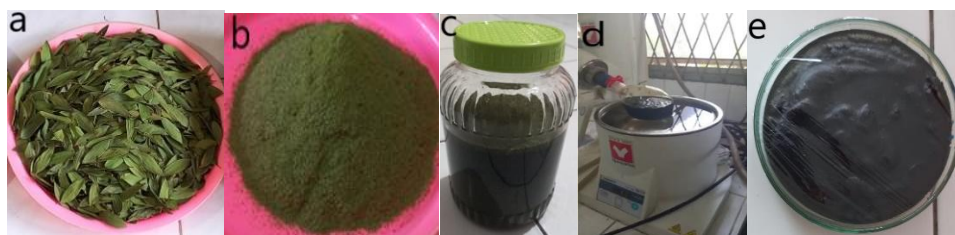


Figure 1. Extraction process of *Melaleuca leucadendra* (a) *Melaleuca leucadendra* leaves; (b) crude drugs; (c) maserate; (d) solvent evaporation; (e) crude extract

Phytochemical screening indicated that ethanol extracts of *Melaleuca leucadendra* leaves contain various secondary compounds, including alkaloids, flavonoids, saponins, tannins, phenolics, terpenoids, and steroids (Table 1). These findings are consistent with previous studies on species in the *Melaleuca* genus, which have been reported to produce bioactive compounds with significant pharmacological activity [16,17,39]. This diversity of phytochemical

classes may indicate that the antibacterial effects of the extract are due to synergistic interactions between different bioactive molecules. Qualitative phytochemical analysis of ethanol extracts from *Melaleuca leucadendra* revealed the presence of several classes of secondary metabolites. Table 1 summarizes the results of colorimetric tests used to detect alkaloids, flavonoids, saponins, tannins, phenolics, terpenoids, and steroids.

Table 1. Phytochemical analysis of ethanol extract from *M. leucadendra* leaves

No	Phytochemical Group	Test Method/Reagent	Color in change	Results
1	Phenolics	FeCl ₃	Blue	[+]
2	Alkaloids	Mayer's/ Dragendorff's	White sediment	[+]
3	Flavonoids	Mg powder + HCl	Orange	[+]
4	Saponins	Foam test	Stable foam	[+]
5	Tannins	FeCl ₃	Blue-black	[+]
6	Terpenoids	Liebermann–Burchard	Reddish brown	[+]
7	Steroids	Liebermann–Burchard	Red	[+]

Notes: "+": presence, "-": absence

Flavonoids and phenolic compounds, detected in phytochemical screening, are known to have antibacterial effects by disrupting microbial cell membranes [29], forming complexes with bacterial proteins, and inhibiting nucleic acid synthesis. Tannins can inactivate microbial adhesins, enzymes, and cell envelope transport proteins, while saponins increase membrane permeability by interacting with sterols, causing cell lysis

[17,40]. The presence of terpenoids and steroids can further enhance antibacterial potential by disrupting the lipid bilayer structure and altering membrane fluidity, leading to increased membrane permeability and leakage of intracellular components [23]. The combination of these mechanisms may explain the broad-spectrum activity observed against Gram-

positive and Gram-negative bacteria in this study.

Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

TFC and TPC were measured at a temperature of 24.6°C with a humidity of 61%, a maximum wavelength of 795 nm for TFC and 430 nm for flavonoids. **Figure 1** shows the absorbance of Quercetin and gallic acid standards for TFC and TPC, with

$R^2 = 0,997$ (TFC) and $R^2 = 0,999$ (TPC). Based on the analysis results, TFC was 17.78 mg QE/g extract, while TPC was 292.43 mg GAE/g extract. These results indicated that the extract is rich in non-flavonoids phenolic compounds. Phenolic compounds have significant antioxidant activity and can support antibacterial properties. Thus, these results show that the extract has abundant secondary metabolites, especially phenolics, which are relevant to its bioactivity potential.

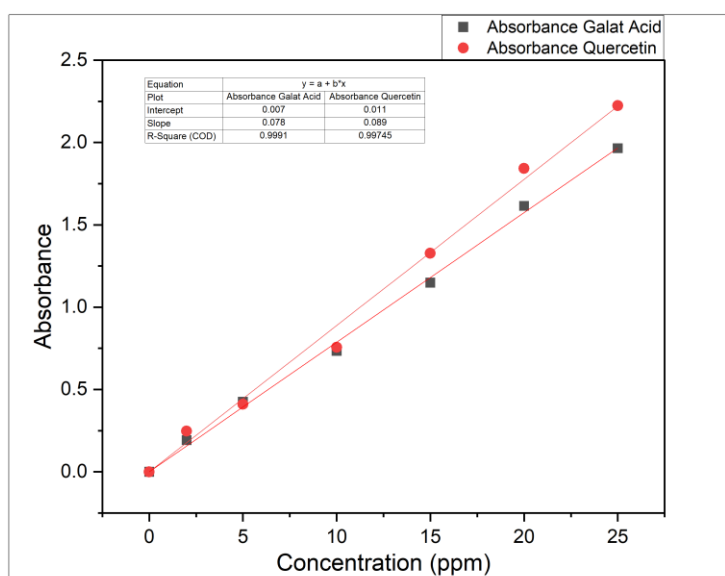


Figure 2. The standard curve of Quercetin and Gallic Acid

In contrast, inhibition efficiency decreased with increasing soaking time, even at constant inhibitor concentrations. This decline can be attributed to the gradual degradation or oxidation of active phenolic compounds during prolonged exposure to the corrosive environment, which reduces their adsorption capability and protective effectiveness. Additionally, extended immersion may lead to partial desorption or deterioration of the inhibitor film, exposing portions of the steel surface to the corrosive medium. Although encapsulation improves the stability of the extract, the results

indicate that its protective performance diminishes over extended soaking periods.

Overall, the findings demonstrate that the corrosion inhibition performance of encapsulated cocoa husk extract is maximized at higher inhibitor concentrations and shorter soaking times. These results confirm the potential of encapsulated cocoa husk extract as an effective and environmentally friendly corrosion inhibitor, while emphasizing the importance of optimizing both concentration and exposure duration for practical applications.

Chromatography–Mass Spectroscopy (GC–MS) Analysis

The GC–MS chromatogram of the ethanol extract of *M. leucadendra* leaves shows several peaks, indicating a complex mixture

of compounds. The retention time, molecular weight, peak area percentage, and reported biological activity of the main constituents are presented in Figure 2 and Table 2.

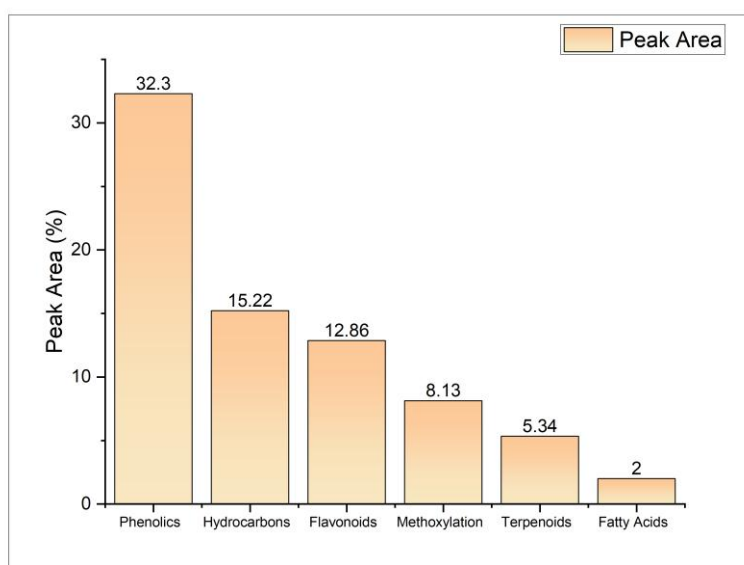
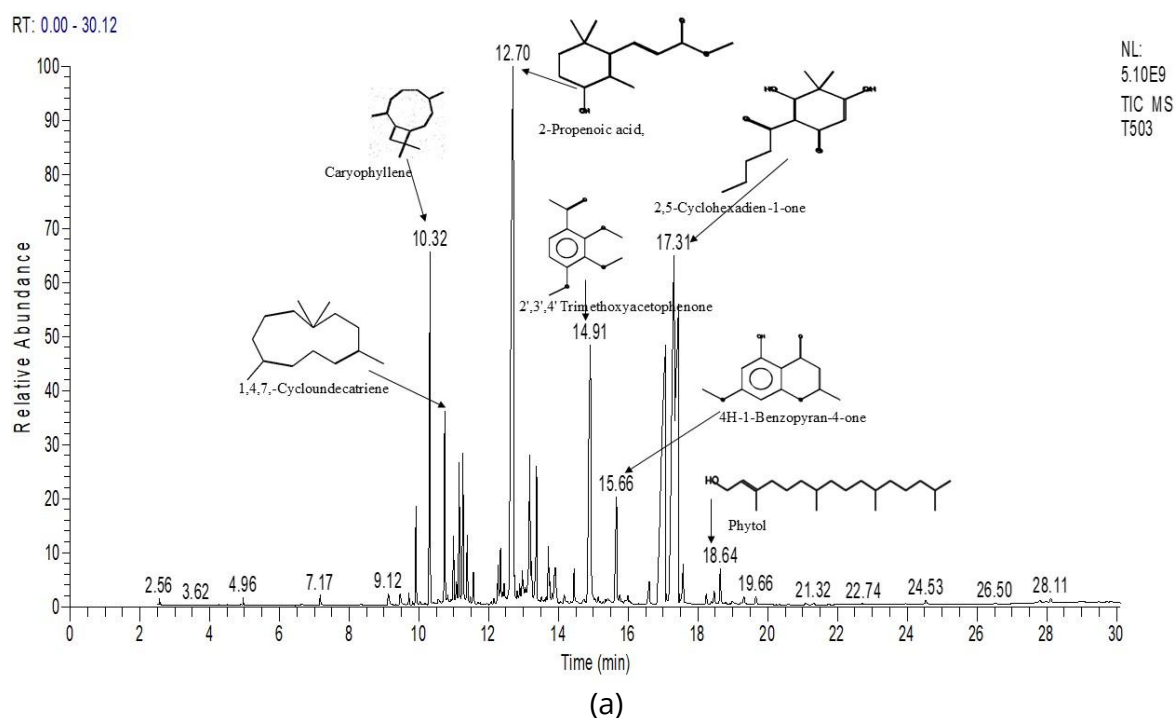


Figure 3. (a) GC–MS chromatogram; (b) compound peak areas of GC–MS analysis of *M. leucadendra* leaves

GC–MS analysis of *Melaleuca sp.* leaf extract shows a wide variety of phytochemicals, which can be classified into several main

groups, including oxygenated phenolics and aromatics, aromatic hydrocarbons, flavonoid compounds, methoxylated

aromatics, terpenoids, and fatty acids and their derivatives Figure 2(b). This chemical distribution confirms the richness of

secondary metabolites in the extract and its potential pharmacological relevance.

Table 2. Major compounds identified in ethanol extracts of *M. leucadendra* using GC–MS

No	RT (min)	Compound Name	Molecular Formula	MW (g/mol)	Peak Area (%)
1	09.92	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	C ₁₅ H ₂₄	204	1.24
2	10.32	Caryophyllene	C ₁₅ H ₂₄	204	5.34
3	10.74	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	C ₁₅ H ₂₄	204	2.59
4	10.99	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	C ₁₅ H ₂₄	204	1
5	11.15	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aà,7à,8aá)]-	C ₁₅ H ₂₄	204	1.89
6	11.26	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2à,4aà,8aá)]-	C ₁₅ H ₂₄	204	2.44
7	12.70	2-Propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-, methyl ester, (E)-	C ₁₃ H ₂₀ O ₃	302	18.86
8	13.18	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1à,4aá,8aà)]-	C ₁₅ H ₂₆ O	222	3.21
9	13.37	Globulol	C ₁₅ H ₂₆ O	222	1.94
10	15.66	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl-	C ₁₁ H ₁₀ O ₄	206	2.20
11	17.06	10-Methylantracene-9-carboxaldehyde	C ₁₆ H ₁₂ O	220	15.22
12	17.31	2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl-2-(1-oxopentyl)-	C ₁₆ H ₂₄ O ₅	446	13.44
13	17.44	4H-1-Benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-methyl-2-butenyl)-	C ₂₁ H ₂₆ O ₄	290	12.86

Tabel 2 shows compounds derived from plants identified by GC-MS with a relative peak area greater than 1%. GC-MS analysis of ethanol leaf extracts of *M. leucadendra* revealed a variety of secondary metabolites, including oxygenated phenolic and aromatic compounds, flavonoids, terpenoids, and fatty acids and their derivatives. This group of compounds has been widely documented for its biological activity, particularly in antibacterial mechanisms. Relative peak areas provide valuable indications of the potential contribution of each compound to the overall antibacterial effectiveness of the extract.

The main compound identified was 2-propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-, methyl ester (18.86%), an oxygen-containing terpenoid ester derivative. Compounds in this class are often associated with membrane-damaging properties, increasing the permeability of bacterial cell walls, and thus disrupting microbial viability [41][42]. The dominance of these compounds suggests that these metabolites may play a major role in the antibacterial activity observed in the extract. Terpenoid esters are classified as phenolic compounds.

Other phenolic compounds detected in relatively high concentrations were phenolic compounds derived from 2,5-Cyclohexadien-1-one (13.44%). The total phenolic compounds detected reached 32.3%, with the main compound being 2-propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-, methyl ester. The oxygenated aromatic compound 10-methylanthracene-9-carboxaldehyde was detected at 15.22%. Phenolic groups are known for their ability to inactivate bacterial enzymes through hydrogen bonding and covalent interactions, while aromatic aldehydes can exert antimicrobial effects through protein denaturation and

disruption of cellular signaling pathways [43]. Overall, these two groups of compounds contributed >40% of the total peak area, highlighting their significant potential in mediating antibacterial effects.

The detection of flavonoid compounds such as 4H-1-benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-methyl-2-butenyl)- (12.86%) further strengthens the biological activity of the extract. Flavonoids have been widely reported to interfere with nucleic acid synthesis, inhibit bacterial topoisomerase, and damage the cytoplasmic membrane [44][45]. The relatively high flavonoid content indicates that flavonoids can act synergistically with phenolic compounds in enhancing antibacterial potential.

Terpenoids are another major group of bioactive compounds, represented by caryophyllene (5.34%), caryophyllene oxide, globulol. Spathulenol and phytol were detected, but with a relative peak area of less than 1%. Terpenoids are known to integrate into the lipid layer, disrupting the stability of bacterial membranes and interfering with respiration [46]. Previous studies have demonstrated the strong antibacterial effects of sesquiterpenes and diterpene alcohols such as phytol against Gram-positive and Gram-negative bacteria. Although their individual peak areas are moderate compared to phenolics, their cumulative abundance is prominent and likely contributes significantly to the antimicrobial activity of the extract.

Fatty acids and their derivatives, including palmitic acid and methyl oleate, were also detected at < 2%. These compounds generally have weaker antibacterial effects than phenolics and terpenoids, but can act synergistically by disrupting membrane integrity and facilitating the activity of more potent metabolites. Synergism between

fatty acids and phenolic/terpenoid constituents has been documented, whereby fatty acids increase permeability and enhance the effectiveness of other antibacterial agents [47,48].

The peak area results of the GC-MS analysis in Figure 3(b) show that the detected secondary metabolites are dominated by phenolic compounds with the highest peak area percentage of 32.3%, followed by hydrocarbons (15.22%) and flavonoids (12.86%), while the compound with the lowest contribution is fatty acids (2%). The dominance of phenolic and flavonoid compounds indicates strong antibacterial activity potential, given that both groups of compounds are known to have antimicrobial properties [49,50] through mechanisms of growth inhibition and damage to bacterial cell membranes. The presence of terpenoids and methoxylated derivatives, although in lower amounts, also enhances the bioactive activity of the extract, while the hydrocarbon and fatty acid content acts as compounds that support stability and solubility [51]. This profile overall confirms that the tested extract has significant pharmacological potential, particularly as a candidate source of natural antibacterial agents.

Overall, the GC-MS profile indicates that the antibacterial potential of *M. leucadendra* extract is not due to a single compound, but rather to complex interactions between various secondary metabolites. The high content of oxygenated aromatic compounds and flavonoids highlights their central role, while terpenoids and fatty acids may provide supporting or synergistic effects. These findings are consistent with previous reports that plant extracts exhibit antimicrobial activity through the combined action of diverse chemical constituents, frequently having effects that are stronger than the sum of the effects of the individual substances.

The data show that ethanol extracts of *M. leucadendra* leaves contain a rich composition of antibacterial metabolites, with phenolics, flavonoids, methoxylated aromatics, and terpenoids as the main contributors. This multi-component profile provides a rational basis for the broad-spectrum activity observed against *S. aureus* and *E. coli* bacteria. Future studies should focus on fractionation, bioassay-guided isolation, and determination of minimum inhibitory concentrations (MIC) to confirm the individual and synergistic contributions of these compounds.

Antibacterial Activities

Antibacterial analysis was conducted on *S. aureus* and *E. coli* with extract concentrations of 10%, 20%, 40% and 80%, to identify the extract with the highest inhibition zone. The extract with a concentration of 80% showed the greatest antibacterial activity with an inhibition percentage of 15.67 ± 0.58 mm against *S. aureus* and 15.33 ± 0.58 mm against *E. coli*. The antibacterial inhibition zones of the ethanol extract of *M. leucadendra* leaves are shown in Table 3 and Figure 3.

Antibacterial activity testing showed a concentration-dependent increase in the inhibition zone, with the 80% extract generating the highest antibacterial activity (Figure 3). This pattern is consistent with the dose-response principle, whereby higher concentrations increase the availability of active compounds to diffuse into the agar medium. Figure 3 shows that the ethanol extract of *M. leucadendra* tested had antibacterial activity against *S. aureus* and *E. coli* with an increase in the diameter of the inhibition zone as the extract concentration increased. At low concentrations (10–20%), *E. coli* has higher sensitivity than *S. aureus*, as seen from the larger inhibition zone, but at the highest concentration (80%), the

inhibition zone against *S. aureus* slightly exceeded that of *E. coli*, indicating the extract's effectiveness against both types of bacteria. This pattern indicates the presence of strong broad-spectrum antibacterial

properties, consistent with the content of secondary metabolites such as phenolics and flavonoids detected through GC-MS analysis.

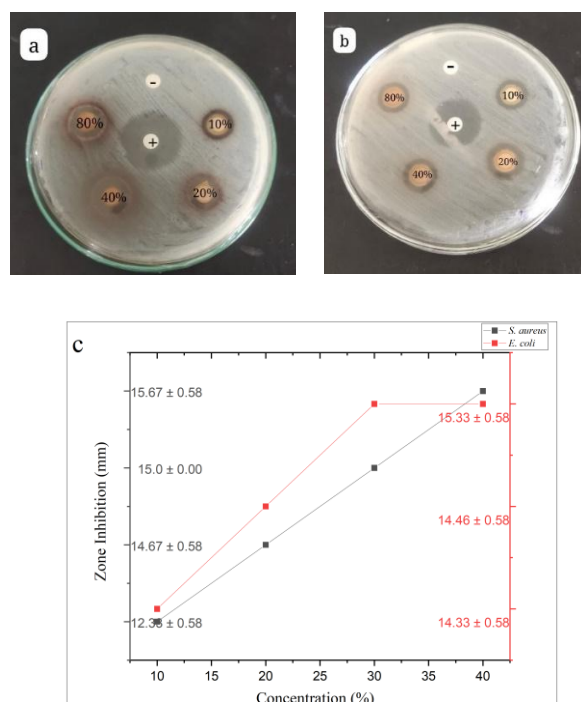


Figure 4. Antibacterial activity of ethanolic extract of *M. leucadendra* leaves: (a) *S. aureus*; (b) *E. coli*; (c) Inhibition zone

Table 3. Inhibition zones of ethanolic extract of *M. leucadendra* leaves against test bacteria

Concentration (%)	Inhibition Zone (mm) and antibacterial power (%) ^a	
	<i>S. aureus</i>	<i>E. coli</i>
10	12.33 ± 0.58 (52.09) ^{**}	14.33 ± 0.58 (62.30) ^{**}
20	14.67 ± 0.58 (61.98) ^{**}	14.46 ± 0.58 (62.87) ^{**}
40	15.0 ± 0.00 (63.37) ^{**}	15.33 ± 0.58 (66.65) ^{**}
80	15.67 ± 0.58 (66.20) ^{**}	15.33 ± 0.58 (66.65) ^{**}
Tetracycline 30 µg (Positive control) ^b	23.67 ± 0.58	23.00 ± 1.15
Aquadest (Negative control)	0.00 ± 0.00	0.00 ± 0.00

Note: a: comparison of the inhibition zone of the test sample with the inhibition zone of the positive control: * weak (<50), ** middle (50-70), *** strong (>70) [35]; b: inhibition zone of the positive control

The effectiveness of the antibacterial inhibition zone of the extract is confirmed in Table 3 and Figure 3. These results support the extract's potential as a naturally occurring antibacterial agent with dose-responsive efficacy.

The antibacterial assay of the extract demonstrated inhibitory activity against both *S. aureus* and *E. coli*, with inhibition zones ranging from 12.33 ± 0.58 mm to 15.67 ± 0.58 mm and 14.33 ± 0.58 mm to 15.33 ± 0.58 mm, respectively (Table 3). When compared to the positive control (tetracycline, 23-23.67 mm), the extract exhibited moderate antibacterial activity, while the negative control produced no inhibition zones, confirming that the observed activity was attributed to the phytochemicals present in the extract.

The antibacterial power values, expressed as percentages relative to the positive control, ranged from 52.09% to 66.20% against *S. aureus* and 62.30% to 66.65% against *E. coli*. These values indicate that the extract possesses a broad-spectrum antibacterial effect, albeit with lower potency compared to standard antibiotics. Interestingly, the inhibitory activity plateaued beyond 40% concentration, suggesting a saturation effect in which further increases in extract concentration did not significantly enhance antibacterial efficacy.

The slightly higher inhibition zones observed against *E. coli* at lower concentrations indicate that the extract may contain bioactive compounds with stronger activity toward Gram-negative bacteria. This is noteworthy, as gram-negative bacteria generally exhibit higher resistance to plant-derived antimicrobials due to their outer membrane barrier. The activity against both gram-positive and gram-negative bacteria suggest that the extract metabolites capable

of disrupting bacterial membranes or interfering with intracellular processes.

Overall, these findings support the potential application of the extract as a natural antibacterial agent. However, its lower efficacy compared to tetracycline highlights the need for further studies focusing on fractionation, isolation of active compounds, and evaluation of their mechanism of action. Additionally, testing against multidrug-resistant bacterial strains may further establish the clinical relevance of the extract.

Raman Spectroscopy

The Raman spectrum in Figure 4 shows a comparison between CS/PVA (black line) and CS/PVA/ML (red line) solutions. The CS/PVA solution is the base solution before the addition of extract, while CS/PVA/ML is the CS/PVA solution to which *Melaleuca leucadendra* extract has been added. In general, both spectra display characteristic bands that indicate the presence of the main functional groups that make up chitosan and PVA, but there is a shift in intensity and position of certain peaks after the addition of *M. leucadendra* (ML) extract, which indicates the presence of molecular interactions between the bioactive compounds from the extract and the polymer matrix. The CS/PVA spectrum shows several characteristic bands, including ~ 268 cm^{-1} and ~ 500 – 600 cm^{-1} : C–C–O bond vibrations from the chitosan polysaccharide structure; asymmetric stretching vibrations of the C–O–C ether group, originating from the main chain of PVA and the glycosidic units in chitosan at ~ 892 cm^{-1} ; weak to moderate bands related to $-\text{NH}_2$ bending and C=C stretching vibrations, indicating the presence of chitosan amine groups and double bonds from PVA at ~ 1460 – 1600 cm^{-1} , indicating carbonyl C=O stretching vibrations, which

may originate from the remaining acetyl group in chitosan or from partial oxidation of PVA $\sim 1720\text{ cm}^{-1}$ (peak shoulder); C-H

stretching bands from both polymers at $\sim 2900\text{--}2950\text{ cm}^{-1}$.

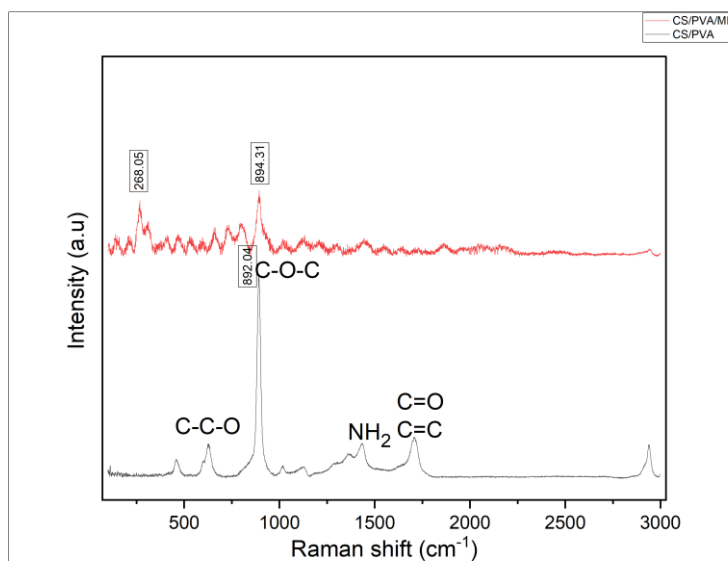


Figure 5. Raman spectroscopy of synthetic polymer solutions for nanofibers

In the CS/PVA/ML spectrum, there are several important changes, namely peaks at 268.05 cm^{-1} and 894.31 cm^{-1} still appear but with higher intensity, indicating the strengthening of C-O-C vibrations due to possible hydrogen interactions between the PVA hydroxyl group and phenolic compounds from the ML extract; there is a small shift in the peak position from $892.04 \rightarrow 894.31\text{ cm}^{-1}$, indicating a change in the chemical environment of the ether group due to the formation of new hydrogen bonds; the band in the $1500\text{--}1700\text{ cm}^{-1}$ region appears to weaken or shift, indicating that the C=O or NH_2 groups of chitosan interact with active compounds such as flavonoids, terpenoids, and fatty acids [52] present in *M. leucadendra* extract; The overall intensity of the CS/PVA/ML spectrum is weaker than that of CS/PVA, indicating an increase in the degree of amorphousness due to complex intermolecular interactions that disrupt the crystallinity of the polymer matrix [53]. Changes in the position and intensity of the Raman bands indicate that the active compounds in the gelam leaf

extract were successfully integrated into the CS/PVA matrix through non-covalent interactions, particularly hydrogen bonds between the -OH or -NH_2 groups of chitosan/PVA and the phenolic groups (aromatic -OH) of the extract.

Raman spectroscopy results show that the solution was successfully formulated through mixing, resulting in the potential for increased release of bioactive compounds and strengthening the antibacterial properties of the material. This modification has the potential to increase the antibacterial activity and biophysical properties (such as permeability and flexibility) of the fibers.

Conclusions

The ethanol leaves extract of *Melaleuca leucadendra* contains various secondary bioactive compounds, including phenolics, flavonoids, saponins, tannins, terpenoids, steroids, and alkaloids. Quantitative studies showed total flavonoids of 17.78 mg QE/g extract, while total phenolics of 292.43 mg

GAE/g extract. GC–MS analysis confirmed the dominance of oxygenated esters, oxygenated aromatics, phenolic derivatives, and flavonoids, which together accounted for more than 40% of the total chemical composition. This group of compounds is known to have antibacterial activity, working through mechanisms such as membrane destabilization, protein binding, and inhibition of enzymatic and genetic processes. Antibacterial testing showed that the extract has an inhibitory effect on *Staphylococcus aureus* and *Escherichia coli*, with the inhibition zone increasing proportionally with concentration. The extract exhibits broad-spectrum activity, demonstrating its potential against both Gram-positive and Gram-negative bacteria. Raman spectroscopy confirmed the interaction between polymer molecules and secondary metabolite compounds in the extract, thereby potentially enhancing antibacterial properties. These findings demonstrate the antibacterial potential of *M. leucadendra*, warranting further studies to identify active compounds and evaluate their individual and synergistic effects through MIC analysis

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Author Contributions

The contribution of each author to this article is: Conceptualization, Atina and Fitri Suryani Arsyad; Methodology, Atina dan Fitri Suryani Arsyad; Formal Analysis, Idha Royani, Assaidah dan Fitri Suryani Arsyad;

Writing – Original Draft Preparation, Atina; Writing – Review & Editing, Idha Royani, Assaidah dan Fitri Suryani Arsyad; Supervision, Dedi Setiabudidaya dan Miksusanti.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Standards

This article does not contain any studies involving human or animal subjects.

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